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90-Day Inhalation Toxicity Study of Hydroprocessed Esters and Fatty Acids (HEFA) Bio-Based Jet Fuel in Rats with Neurotoxicity Testing and Genotoxicity Assay

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14. ABSTRACT
The Department of Defense is actively pursuing the development of alternative fuels to augment or replace petroleum-based jet fuels. All new fuels are potentially hazardous to Air Force personnel and require toxicity evaluation. Hydroprocessed Esters and Fatty Acids jet fuel (HEFA) is a type of hydrotreated renewable jet fuel currently under consideration. One specific type of HEFA is generated from oils extracted from the camelina plant (*Camelina sativa*; HEFA-C). In order to evaluate potential toxicity of HEFA-C, an *in vivo* 90-day whole body inhalation study was performed with the fuel (concentrations of 0, 200, 700 and 2000 mg/m^3 for 6 hours/day, 5 days/week) using male and female Fischer 344 rats. There was no change in food consumption attributed to fuel exposure and the average body weight was found to slightly decrease (not statistically significant) in animals exposed to the high concentration. Micronucleus test was negative for evidence of genotoxicity. No significant effects were observed for clinical chemistry or hematology analyses and no significant neurobehavioral effects were observed based on functional observational battery and motor activity tests. Minimal effects attributable to HEFA-C exposure were observed with histopathology. These effects included goblet cell hyperplasia of nasal epithelium and olfactory epithelium degeneration at the highest concentration of exposure. These two nasal cavity locations were concluded to be the primary target tissues for HEFA-C in this 90-day study; HEFA-C toxicity overall was less than the current jet fuel, JP-8.

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PREFACE

Funding for this project was provided through the Alternative Fuels Certification Office (AFLCMC/WNN). This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF). The program manager for the contract was David R. Mattie, PhD (711 HPW/RHDJ), who was also the technical manager for this project. The study was conducted under Navy work unit number H1104 under the management of NAMRU-Dayton.

The 90-day study protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (U.S. EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870.3465: 90-Day Inhalation Toxicity (1998) and the Organisation for Economic Co-operation and Development Guidelines for Testing of Chemicals, Test No. 413, Subchronic Inhalation Toxicity: 90-day Study (OECD, 2009). The neurotoxicity portion of the study follows OPPTS 870.6200 Neurotoxicity Screening Battery (1996a) and the genotoxicity portion follows OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test (1996b).

This study was not performed in a Good Laboratory Practice (GLP) Standards certified laboratory, and therefore there is no certification of compliance with GLP regulations (40 CFR Part 792). However, this study was conducted with an effort to follow the intent and purpose of GLP requirements.

The 90-day study with Neurotoxicity Testing and Genotoxicity Assay was approved by the Wright-Patterson Air Force Base (AFB) Installation Animal Care and Use Committee (IACUC) as protocol number F-WA-2011-0126A. The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

The authors would like to acknowledge LTC Deidre Stoffregen (DVM, DACVP, Veterinary Corps, U.S. Army, 711th Human Performance Wing (711 HPW/RHDV), Wright-Patterson AFB OH) for conducting gross pathology observations.

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1.0 SUMMARY

Male and female F-344 rats were exposed by inhalation to an aerosol and vapor of HEFA-C fuel. Animals were exposed to three target concentrations, 200, 700 and 2000 mg/m^3, and a control exposure to clean air. A total of ten male and ten female rats were exposed at each concentration, divided into two replicates with five males and five females each. The replicates were staggered by one day at the start of exposures. Exposures were conducted for six hours/day, five days/week. Each rat incurred a total of 71 exposure days.

Animals were assessed for neurobehavioral function via a functional operational battery (FOB) after the 13th week, and motor activity after the 14th week. There were no significant observations relatable to exposure to HEFA-C fuel in either the FOB or motor activity measurements.

Animals were necropsied the day after the last exposure. Blood samples were taken for clinical chemistry and hematology, and the animals were examined for gross lesions. There were no gross lesions relatable to HEFA-C exposure. Target tissues were examined histopathologically. No adverse effects were seen in any target tissues other than the nasal tissues. Olfactory epithelial degeneration and goblet hyperplasia was observed in the nasal turbinate airways of the high concentration (2000 mg/m^3) male and female rat.

Animals exposed to the high concentration of HEFA-C fuel showed somewhat lower body weights. In the high concentration (2000 mg/m^3) group, the average male body weight was decreased by approximately 5 percent, while the average female body weight decreased by 3 percent by the end of the exposures. These changes were not statistically significant. Differences in the average organ weights were not significant except for a spleen and liver weight increase in the intermediate concentration exposure females. The reason for the weight increase is unknown, and did not demonstrate a dose-response relationship with exposure to HEFA-C fuel. There were some statistically significant differences in the clinical chemistry and hematology that were not considered to be biologically significant. The alpha 2-microglobulin protein results showed a slight increase in measured levels relative to exposure concentration. The histopathology did not see any significant difference in hyaline droplets in the male rat kidney in any concentration group compared with the controls. No significant differences were observed for the male reproductive endpoints of interest. The vaginal cytology results did not show a significant alteration of the estrus cycle.

In conclusion, the primary target organ for HEFA-C jet fuel appears to be the nasal tissues. In the nasal cavities, the olfactory epithelial degeneration and goblet hyperplasia of nasal epithelium were seen at the 2000 mg/m^3 dose. Overall, toxicity was less than the current jet fuel, JP-8.

2.0 INTRODUCTION

The Office of the Secretary of Defense Assured Fuels Initiative is examining alternative fuels for military use to decrease dependence on foreign oil sources (Blackwell, 2007). These fuels would potentially be used in military aircraft, ships, and ground vehicles. Fuels are among the most common sources of military occupational exposures. Dermal contact and inhalation are generally the primary routes of exposure. During fueling operations, personnel may be exposed to both vapors and aerosols of jet fuel by inhalation. Preliminary analysis of the new fuels shows that many of the ingredients are the same as JP-8, the traditional military fuel, but the composition is still different in each fuel. Therefore, the health effects associated with exposure to each alternative fuel may be significantly different than JP-8 and should be examined by relevant routes of exposure.

An alternative fuel previously tested for toxicity is a synthetic jet fuel produced by the Fischer-Tropsch (F-T) process, that is referred to by the Air Force as Synthetic Paraffinic Kerosene (SPK) (Hinz *et al.*, 2012; Hurley *et al.*, 2011; Mattie *et al.*, 2011a, 2011b, 2011c; Riccio *et al.*, 2010). Biobased jet fuels are also undergoing development and certification for use by the military. Each new fuel needs to be examined for their toxicity potential compared to JP-8 and F-T. One source of biofuels is from Hydroprocessed Esters and Fatty Acids, or HEFA. Fuels from this process were previously called hydro-treated renewable jet (HRJ) fuel. In this process, the oil is treated by a process of hydrogenation/de-oxygenation of free fatty acids to convert triglycerides to synthetic paraffinic kerosene suitable for use as a jet fuel. One of the HEFA biofuels is based on oils extracted from the camelina plant (*Camelina sativa*) and is known as HEFA-C.

Very limited toxicity testing of HEFA-C jet fuel has been performed. Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of HEFA-C by inhalation is needed to assess the risk of replacing or augmenting JP-8 with this fuel.

2.1 Objective

The objective of this study was to assess the potential toxicity of the HEFA-C fuel by inhalation. Fischer 344 Rats were exposed by inhalation to an aerosol and vapor mixture of HEFA-C jet fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (control), 200, 700 or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. Neurotoxicity and genotoxicity endpoints were also assessed.

3.0 METHODS

3.1 Study Design

This study was designed to assess the potential inhalation toxicity of HEFA-C fuel when administered as an aerosol and vapor via inhalation exposure to rats for 6 hours per day, 5 days per week over approximately 90 days, at concentrations of 0, 200, 700 and 2000 mg/m^3. Ten male and ten female Fischer-344 rats were in each exposure group, and each exposure group had two replicates of five males and five females each (Table 1). The replicates were staggered by one day in the exposure schedule to accommodate the necropsy at the end of exposures. Due to the stagger in exposures, accommodation of holidays and the neurobehavioral test (functional observation battery, FOB) schedule, the study spanned a total of 107 days from first exposure to last necropsy (for a nominal 90-day study), with each replicate receiving 71 exposures (Table 2). After the completion of exposures, animals were euthanized and necropsied. Assessments included clinical observations, gross pathology, histopathology of target organs, FOB and motor activity tests, vaginal cytology and sperm morphology evaluations. The study protocol can be found in Appendix A.

Table 1: Study Design

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control			
Replicate 1	0	5	5
Replicate 2	0	5	5
Low Group			
Replicate 1	200	5	5
Replicate 2	200	5	5
Intermediate Group			
Replicate 1	700	5	5
Replicate 2	700	5	5
High Group			
Replicate 1	2000	5	5
Replicate 2	2000	5	5
Total		40	40

Table 2. Timeline of In-life Activities

Date	Activity
April 8, 2011	Animals ordered
April 19, 2011	Animals arrive, placed in room 108
May 4, 2011	Animals begin cage acclimation
May 6, 2011	Pre-study ophthalmologic exam conducted
May 11, 2011	Exposures begin for Replicate 1
May 12, 2011	Exposures begin for Replicate 2
May 30, 2011	Holiday, no exposure
July 4, 2011	Holiday, no exposure
July 25-29, 2011	Vaginal cytology conducted
August 11, 2011	Motor activity measurements conducted with Replicate 1
August 12, 2011	Motor activity measurements conducted with Replicate 2
August 18, 2011	Replicate 1 FOB conducted
August 19, 2011	Replicate 2 FOB conducted
August 11 & 12, 2011	Final ophthalmologic exam conducted
August 24, 2011	Last exposure
August 24, 2011	Replicate 1 necropsy
August 25, 2011	Replicate 2 necropsy

3.2 Test Substance

The HEFA-C jet fuel was obtained from the manufacturer (UOP LLC, a Honeywell Company, Des Plaines IL) by the Air Force Research Laboratory (AFRL/RQTF) Fuels Branch at Wright Patterson Air Force Base (AFB) OH. The method of synthesis of HEFA-C jet fuel is maintained by the manufacturer. An additive package consisting of chemicals normally added to JP-8 jet fuel was mixed with the HEFA-C fuel by the Fuels Branch. The combination of HEFA-C fuel with additives was designated as POSF log book number 6152 by the Fuels Branch. Records regarding the receipt of the base HEFA-C fuel and additive package and the POSF log book are maintained by the Fuels Branch. Information regarding the purity, composition and stability of the HEFA-C fuel is maintained by the Fuels Branch. The jet fuel was administered as supplied (POSF 6152). The HEFA-C fuel was stored under room temperature ambient conditions.

3.3 Animals and Animal Husbandry

Animals were ordered and delivered in two batches (Charles River Laboratories, Kingston NY). A total of 80 animals (with 2 additional animals for quality control and sentinel/serology) about 5 weeks old were ordered and received for the 90-day study. A total of 60 animals were ordered and received for the genotoxicity (micronucleus) portion of the study.

3.4 Quarantine and Acclimation Period

Shortly after their arrival at the laboratory, the animals were transported to a room selected for quarantine and acclimation. The animals were removed from the shipping cartons and examined. All animals appeared to be in good condition at receipt. During the quarantine and facility acclimation period, animals were individually housed in solid bottom shoebox-style plastic cages. Towards the end of quarantine and during the week prior to start of exposures, rats were acclimated to the stainless steel wire-mesh Toxic Hazard Research Unit (THRU) cage units in the animal room. Animals were placed in the inhalation wire mesh cages for an increasing length of time (i.e., 1, 2, 3, 4 and 6 hours) on successive days. Prior to group assignment, all animals were examined by an animal care staff member to ascertain suitability for study. No animals were considered unsuitable.

Animal room conditions were maintained at a target of 22 °C, 50 percent humidity, with a 12 hour light/dark cycle. Animals were fed a certified rodent diet (Formulab Diet Purina Lab Chow, PMI Nutrition, International, LLC, Brentwood MO) and reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food and water were unavailable. On the morning of each exposure, animals were transferred from their home caging to the THRU cage units. After the exposure was finished for the day, animals were transferred back to their home shoebox-style caging.

Body weights were measured the day after arrival. During the acclimation period, animals were randomly assigned to the exposure groups. Individual weights of animals placed on test were within ± 16 percent of the mean weight for each sex at the start of the study. To make food consumption measurements, feeder weights were measured periodically.

3.5 Ophthalmologic Exam

Animals were given an ophthalmological exam to look for eye defects prior to and at the end of the study. An ophthalmoscope was used in addition to a gross observation of the eyes.

3.6 Exposure System

Rats were exposed by inhalation in a THRU chamber with a volume of 690 L. Four chambers were used, one for each exposure group. The chambers have a maximum capacity of 32 rats held in THRU stainless steel wire mesh cage units. The THRU exposure chambers were operated at a flow rate of approximately 180 L/min to provide at least one complete air change in 3.8 minutes (15.8 air changes/hour); minimum guideline requirements are 10 air changes per hour. The chambers had a T_{99} equilibrium time of approximately 18 minutes; T_{99} is the time for the concentration of test substance in the chamber to rise from background or zero to 99 percent of the equilibrium or target concentration. This chamber size and airflow rate was considered adequate to maintain an oxygen level that is at least 19 percent, the minimum required by the guidelines. At the end of an exposure, the chamber was operated at approximately the same flow

rate using clean air for approximately 30 minutes so that the bulk of the test material was cleared from the chamber before removing the animals.

Air from the room was passed through a 95 percent high efficiency particulate air (HEPA) filter and distributed by a blower to the exposure chambers. Air flow was measured by monitoring the pressure drop across a laminar flow element at the inlet to each chamber. The temperature in each chamber was monitored using a type J thermocouple. Relative humidity in the control chamber was measured by using a humidity temperature transmitter (Omega Engineering, Inc., Stamford CT).

3.7 Generation System

Jet fuel exposure atmospheres were generated as a mixture of aerosol and vapor by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton IL). A liquid metering pump (Fluid Metering, Inc. (FMI), Syosset NY) pumped liquid jet fuel from a glass bottle reservoir to the nozzle. Compressed instrument air at approximately 50 psi was supplied to the nozzle. The spray was directed into a custom-made PVC mixing volume and then injected into the inlet air stream to the exposure chamber.

3.8 HEFA Concentration Measurement

A Fourier transform infrared (FTIR) spectrophotometer (Nicolet Model 380, Thermo Scientific, Waltham MA) was used to monitor the concentration of jet fuel in the chamber. A sample of the chamber atmosphere was pulled through the FTIR spectrophotometer. The signal output from the FTIR was recorded by computer. The infrared spectrophotometer was calibrated by injecting a known volume of jet fuel into a Tedlar ® (DuPont, Wilmington DE) bag filled with a known volume of air and sampling with the FTIR. A calibration curve of spectrophotometer response as a function of jet fuel concentration was produced.

Nominal concentration was calculated from the air flow rate through the chamber, the total generation time, and the mass of fuel consumed during the exposure.

3.9 Necropsy

Following the last exposure, animals were weighed and then euthanized by an overdose of sodium pentobarbital. Blood was collected via the caudal vena cava for hematology and clinical chemistry. Following the blood sample, the rat was rapidly decapitated with a rat guillotine. The left testicle and epididymis were taken for sperm analysis, and then necropsied for the remaining tissues. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Pathology observations were conducted by a board certified veterinary pathologist. Wet weights of the liver, kidneys, adrenals, testes, epididymides, ovaries, uterus,

thymus, spleen, brain and heart were obtained after dissection from the exposure and control animals.

3.10 Clinical Chemistry and Hematology

During necropsy, blood samples were taken for measurement of clinical chemistry and hematology endpoints. One whole blood sample was analyzed immediately for clotting time tests. Prothrombin time (PT) and international normalized ratio (INR) were determined using a blood clot analyzer (GEM PCL Plus, Instrumentation Laboratory, Lexington MA). Samples of whole blood with anticoagulant were examined using a blood analyzer (Hemavet 950, Drew Scientific, Dallas TX), while samples of plasma were assessed using a chemistry analyzer (Vet Test 8008 and Vet Lyte, IDEXX Laboratories, Westbrook ME).

3.11 Measurement of Alpha 2-Microglobulin Protein

Kidneys from male and female rats were removed and one-half of each kidney (left cut longitudinally, right cut transversely) was flash frozen and stored at -80 °C until use. An enzyme-linked immunosorbent assay **(**ELISA) procedure was used with kidney homogenates by standard techniques to quantify the amount of alpha 2-microglobulin protein in kidney tissue.

3.12 Sperm Motility and Concentration

For a male rat, the right epididymis was immediately dissected out and weighed. The cauda was then removed, the remainder of the epididymis weighed again and frozen for future analysis. The cauda was submitted for sperm motility analysis. For the males necropsied on the first day and a portion on the second day, the caput (head) of the epididymis was taken instead of the cauda as the source for sperm for motility analysis. The cauda was taken for the last eight males (two per exposure group) necropsied on the second day.

3.13 Vaginal Cytology

Vaginal cytology was conducted on all female rats. During the 12^{th} week of exposure (July 25 to July 29, 2011), a vaginal lavage was performed on each female rat daily, prior to exposure, over the five-day period. Approximately 10 µL of physiological saline was gently flushed into the vaginal opening and aspirated back into a pipette tip. The aspirate was placed a glass slide. The slides were prepared and read using light microscopy.

3.14 Motor Activity

Gross locomotor movements and exploratory behavior (e.g., motor activity) were evaluated in the animals after about 64 days of exposure, using a photobeam activity system (PAS) and

software (San Diego Instruments, San Diego CA). Animals were individually placed in clear plastic open fields (40.6 cm x 40.6 cm x 38 cm, width x depth x height) with horizontal and vertical photobeam frames that automatically recorded beam breaks using the PAS software. The photocells were mounted 1 inch apart in frames placed at ground level to detect horizontal movement and in an elevated frame to detect vertical rears, as well as differentiate small (stereotypic) movements from large movements. Motor activity was measured in a room with white noise generated at 73 dB to mask ambient room levels of ~70 dB and low illuminating light set at 30 lux. An animal was placed in the center of the open field and left uninterrupted for the duration of a 1 hour test session. A computer system automatically recorded all beam breaks.

3.15 Functional Observational Battery (FOB)

Neurobehavioral evaluations were performed using an observation battery designed to detect functional deficits after about 68 days of exposure. On a non-exposure day following three consecutive days of exposures, animals from one replicate group were transferred into the neurotoxicology observation room. The second replicate group was transferred the next day. A FOB consisting of non-invasive procedures designed to evaluate and document the absence or presence (with severity, if appropriate) of a predetermined set of behavioral and clinical signs was performed. Observations are made: 1) while the rat was in an observation cage, 2) during removal of the rat from the observation cage, 3) while the rat was being held and examined for clinical observations, 4) as the animal moved freely about the open field, and 5) during manipulative tests. The observations proceeded from the least to most manipulative tests to reduce the influence of handling on the rat's behavior. Efforts were made to control conditions that could affect behavior, including sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions.

3.16 Micronucleus Genotoxicity Assay

In this study, five males and five female F344 rats per exposure concentration (total of 40 animals) were exposed for two weeks to the HEFA-C fuel (concurrent with the experiment described above) to assess the genotoxic potential of the fuel. An additional 10 males and 10 females, not exposed to jet fuel, were administered positive and vehicle controls, for a total of 60 animals for the micronucleus assay. At the end of exposures, animals were euthanized and dissected to extract bone marrow from the femur. The bone marrow was processed and analyzed for micronuclei by measuring the frequency of micronucleated cells by flow cytometry in approximately 20,000 reticulocytes from each animal. The percentage of reticulocytes, micronucleated mature normochromatic erythrocytes, and micronucleated reticulocytes were determined per animal.

4.0 RESULTS

4.1 Test Substance Characterization

The HEFA-C fuel with additives (POSF 6152) was used as supplied by the Fuels Branch (AFRL/RQTF, Wright-Patterson AFB OH). Information and data regarding the manufacture, composition, physical and chemical characteristics may be obtained from the Fuels Branch.

4.2 Exposure Period

For this study, the exposure period started when the compressed air and the HEFA-C fuel flow were applied to the nozzle. The concentration in the chamber began to increase immediately, as observed on the FTIR. At the end of the exposure period, the compressed air and fuel flow to the nozzle were shut off. Animals were maintained in the chambers with a continuous flow of clean air for approximately 30 minutes. All animals were moved from the exposure chambers to domiciliary housing in the Wright-Patterson AFB vivarium; control animals were housed in a separate room.

4.3 Exposure Conditions

Over the course of the exposures, concentration, temperature, humidity, air flow and static pressure readings were monitored. The average temperature, humidity and air flow remained at target measurements, and did not deviate outside of prescribed ranges. The chamber temperatures were recorded three times per exposure period. The study average temperatures were 22.1 ± 0.7, 22.6 ± 0.7, 22.7 ± 0.6, and 22.3 ± 0.7 °C for the 0, 200, 700 and 2000 mg/m^3 chambers, respectively.

The HEFA-C fuel concentration in chamber was measured continuously by FTIR, and an exposure period average recorded at the end of each daily exposure. The study average of chamber concentrations were 0.9 ± 2.4, 194.8 ± 15.3, 702.5 ± 29.8 and 1990.5 ± 52.4 mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 chambers, respectively. Nominal concentrations, based on the HEFA-C fuel used and the chamber air flow, were 245.9 ± 50.2, 816.5 ± 114.4 and 2411.4 ± 170.2 mg/m^3, giving analytical to nominal concentration ratios of 0.82, 0.87 and 0.83, respectively (Table 3).

A protocol deviation was documented in which the intermediate and high concentration groups of animals were inadvertently switched when placed in the exposure chambers on study day 48, June 27, 2011. Hence, the intermediate group was exposed to the high concentration (2000 mg/m^3) for one day, and the high concentration group was exposed to the intermediate concentration (700 mg/m^3) for one day. The control and low exposure group were unaffected. After that single exposure, animals were placed in the proper chambers for the remaining exposures.

Exposure concentrations in Table 3 are different from the chamber concentration, as they were recalculated to include the deviation day's exposure. As a result, the mean exposure concentration was 2.5 percent higher than the average chamber concentration and 2.8 percent higher than the target 700 mg/m^3 for the intermediate group. For the high exposure group, the mean exposure concentration was 0.9 percent lower than the average chamber concentration and 1.3 percent lower than the target 2000 mg/m^3. A complete report of exposure conditions can be found in Appendix B.

Table 3. Inhalation Atmosphere Summary

	Target Concentration	**0 (mg/m^3)**	**200 (mg/m^3)**	**700 (mg/m^3)**	**2000 (mg/m^3)**
Chamber Temperature (°C)	Mean	**22.1**	**22.6**	**22.7**	**22.3**
	SD	0.7	0.7	0.6	0.7
	N	73	73	73	73
Chamber Concentration (mg/m^3)	Mean	**0.9**	**194.8**	**702.5**	**1990.5**
	SD	2.4	15.3	29.8	52.4
Exposure Concentration (mg/m^3)*	Mean	**0.9**	**194.8**	**719.8**	**1973.2**
	SD	2.4	15.3	154.2	156.4
Gravimetric Concentration (mg/m^3)	Mean	NA	ND	**4.63**	**242.7**
	SD			2.96	34.6
Proportion of Total Concentration				0.007	0.12
Particle Size	MMAD (μm)	NA	ND	**3.06**	**2.60**
	GSD			2.06	1.64

Notes: *Exposure concentrations in the intermediate and high groups differ from the chamber concentrations. These groups were inadvertently loaded into the incorrect chambers for a single exposure. The average concentration experienced by the groups was recalculated to include the one day of the switched exposure concentration.
GSD = geometric standard deviation; MMAD = mass median aerodynamic diameter; N = number; NA = not applicable; ND = aerosol was not detected in the low concentration chamber; SD = standard deviation

The aerosol mass concentration was measured using gravimetric filters. Filter samples were taken during exposures approximately two to three times per week over the course of the study. The average aerosol concentrations were 4.6 ± 3.0 and 242.7 ± 34.6 mg/m^3 for the intermediate and high exposure concentration chambers, respectively (Table 3). No aerosol was detected in the low concentration chamber. The aerosol fraction was 0.7 percent and 12 percent of the total jet fuel concentration in the intermediate and high concentration chambers, respectively. Thus,

as the total HEFA-C fuel concentration increased, the fraction of the total that existed as aerosol droplets increased.

A cascade impactor (In-Tox products, Moriarty NM) was used to measure the particle size distribution. Measurements were made by sampling from a chamber approximately once during a week. The average mass median aerodynamic diameter and geometric standard deviation (MMAD (GSD)) of the aerosols were calculated as 3.06 (2.06) and 2.60 (1.64) µm for the intermediate and high concentration chambers, respectively (Table 3). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents.

4.4 Statistics

Statistical parameters were calculated based on exposure group (and not on replicate groups) using a statistical analysis program (SigmaPlot, v.11.0, Systat Software, Inc., San Jose CA) for in-life data such as body weight, body weight gain and food consumption. Normality was tested using the Shapiro-Wilk test. Levene's test ($p < 0.01$) was performed to check for equal variance. A one-way analysis of variance (ANOVA) ($p<0.05$) was conducted if data passed the tests for normality and equal variance. If means were unequal, then a Holm-Sidak test was used to compare pairs of treatment means ($p >0.05$). A Kruskal-Wallis ANOVA on ranks ($p<0.05$) was run if the normality or equality of variance test failed for a specific parameter.

4.5 Body Weight Results

Individual body weight data may be found in Appendix C. For body weights measured weekly on Wednesday mornings, there was no statistically significant difference between any of the exposure groups and the control groups for either males or females over the course of the study (Tables 4 and 5, Figures 1 and 2). The terminal body weights of animals taken at necropsy were lower than the weights measured in the last week of exposure in part because animals were fasted the evening prior to necropsy. Animals exposed to the high concentration of HEFA-C fuel showed somewhat lower body weights. In the 2000 mg/m^3 exposure group, the average body weights decreased by approximately 5 and 3 percent in the male and female rats, respectively; these changes were not statistically significant.

One set of data requires some explanation; the males and females in the low concentration group showed unusually low body weights on study day 71 (study week 11). The loss of weight prompted a review of the animal housing systems. It was suspected that a water line to the cage rack may have been restricted; however, this could not be confirmed as all water lines were patent upon examination. Weights of animals in the low exposure group returned to anticipated normal over the following two weeks. Animals exposed to the high concentration of HEFA-C fuel showed somewhat lower body weights. In the high concentration (2000 mg/m^3) group, the average male body weight was decreased by approximately 5 percent, while the average female body weight decreased by 3 percent by the end of the exposures.

Table 4. Group Mean Body Weights, Male

Study Day	Control			Low (200 mg/m^3)			Intermediate (700 mg/m^3)			High (2000 mg/m^3)	
-7	178.2	±17.6		185.0	±8.1		172.6	±8.6		171.2	±11.0
1	204.6	±20.9		211.4	±8.7		195.8	±8.9		197.5	±11.7
8	224.9	±22.3		230.4	±7.9		218.7	±13.4		215.3	±12.9
15	240.3	±23.9		247.6	±8.3		232.2	±13.4		227.2	±13.3
22	252.8	±24.3		258.3	±10.2		244.9	±13.5		238.6	±14.0
29	266.9	±24.2		269.2	±12.6		258.4	±12.0		249.9	±16.3
36	280.2	±26.7		281.3	±12.2		269.4	±11.5		262.3	±18.6
43	290.6	±26.7		288.6	±14.2		276.7	±11.1		271.5	±17.3
50	297.6	±26.0		297.0	±13.4		285.8	±9.9		280.7	±19.0
57	307.3	±26.0		308.8	±12.7		298.3	±9.9		293.5	±17.7
64	314.7	±28.0		317.5	±12.1		306.2	±9.8		299.7	±18.4
71	322.3	±28.4		306.5	±11.9		313.9	±10.0		306.2	±17.6
78	329.7	±30.9		322.7	±11.1		320.3	±11.7		312.9	±17.4
85	335.8	±29.0		334.3	±11.9		324.7	±10.9		320.1	±18.9
92	340.0	±29.0		340.0	±12.0		329.0	±13.8		323.5	±18.0
99	344.4	±30.0		342.4	±12.4		334.8	±11.6		327.8	±19.8
106	337.9	±35.1		337.1	±19.2		330.1	±20.2		319.0	±22.1
107	346.8	±35.5		334.2	±10.9		329.2	±12.4		308.6	±23.4

Note: average ± standard deviation, weight in grams

Table 5. Group Mean Body Weights, Female

Study Day	Control		Low (200 mg/m^3)		Intermediate (700 mg/m^3)		High (2000 mg/m^3)	
-7	117.4	±8.2	118.1	±8.3	117.5	±8.2	115.1	±3.4
1	131.9	±9.3	134.8	±9.0	132.7	±7.7	130.2	±3.9
8	140.8	±10.0	145.0	±9.6	142.8	±8.7	138.3	±4.0
15	147.7	±9.9	152.4	±9.3	149.6	±8.3	144.8	±5.1
22	152.8	±10.3	159.2	±8.3	155.4	±9.2	149.8	±3.9
29	158.6	±11.5	163.9	±8.7	159.8	±9.7	154.0	±4.2
36	162.1	±11.3	169.3	±10.4	165.2	±1±1.0	157.4	±4.8
43	166.4	±11.3	171.9	±10.9	168.9	±1±0.6	161.4	±4.6
50	170.4	±12.0	175.6	±9.5	171.5	±±9.5	166.1	±5.3
57	173.0	±10.6	181.2	±8.2	177.3	±1±0.7	172.4	±6.7
64	175.5	±11.9	183.8	±8.2	179.0	±9.3	172.7	±5.3
71	177.5	±11.8	177.8	±7.3	179.7	±9.2	174.7	±5.6
78	179.8	±11.8	183.4	±6.9	183.4	±8.5	175.6	±5.2
85	181.2	±12.0	188.8	±8.0	186.1	±9.7	175.8	±6.3
92	184.6	±12.1	189.8	±7.8	188.5	±9.0	179.2	±5.5
99	185.3	±11.6	192.8	±9.2	189.4	±10.5	180.6	±7.0
106	180.8	±10.6	187.4	±8.1	187.7	±8.8	175.3	±10.8
107	173.0	±6.8	180.8	±9.3	178.8	±9.8	174.4	±9.2

Note: average ± standard deviation, weight in grams

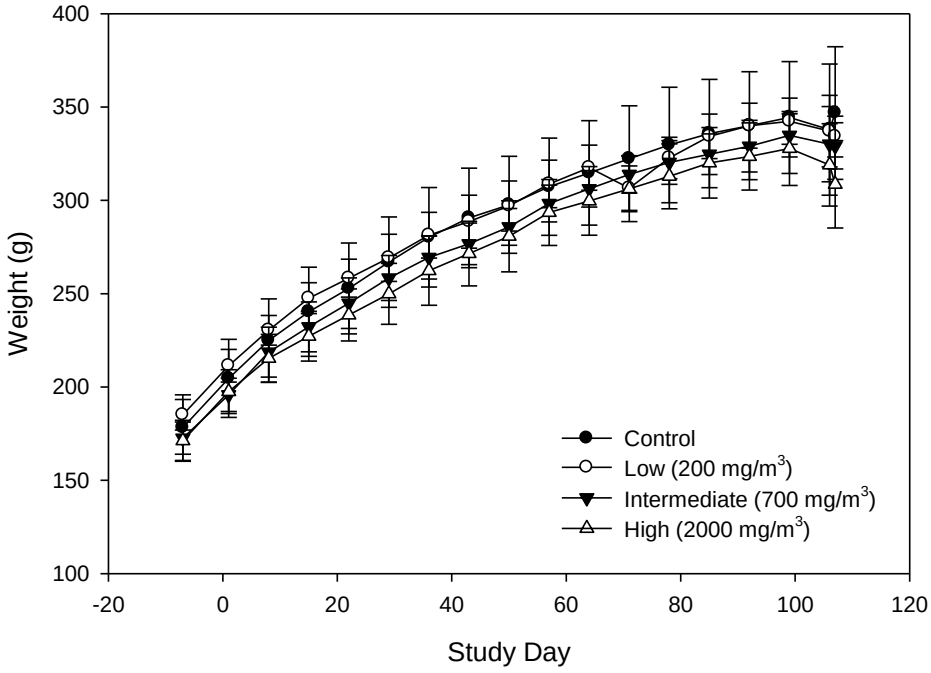

Figure 1. Group Average Body Weights, Male. Shapes indicate weekly group average in grams; bars indicate standard deviation.

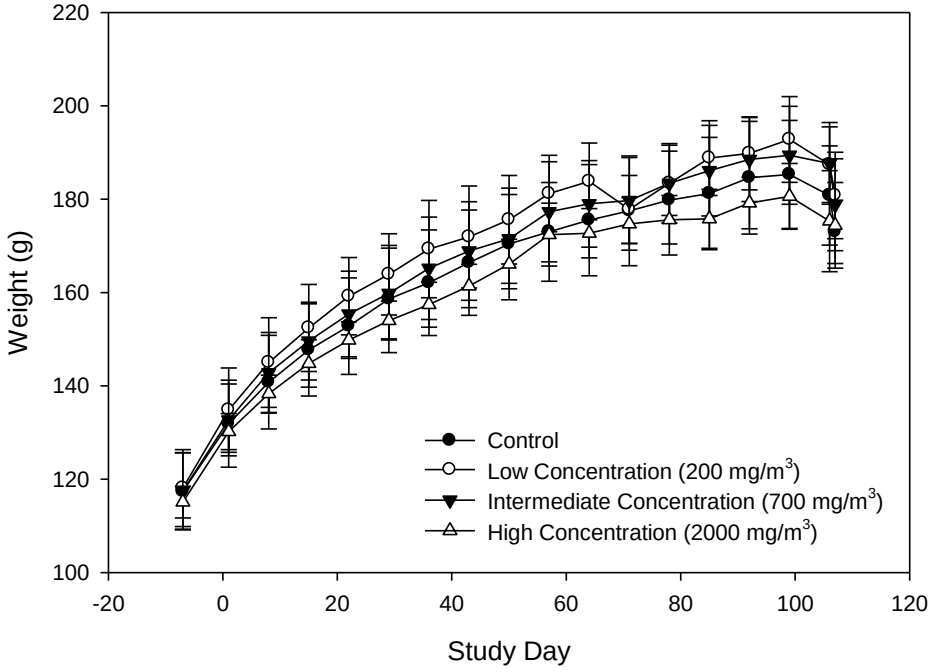

Figure 2. Group Average Body Weights, Female. Shapes indicate weekly group average in grams; bars indicate standard deviation.

While all exposed groups were not statistically different from controls, both males and females in the high dose group demonstrated a trend of decreased body weight compared with controls. Male average weights in the high concentration group tended to average lower than control weights. In the final week of exposures, the group average weight of the high exposure group males was approximately 5 percent lower than controls. In females, the high exposure concentration group consistently had a lower group average weight than controls. During the last week of exposures, the high concentration female average body weight was about 3 percent lower than controls.

4.5.1 Body Weight Gain. Body weight gains were calculated based on the body weight measurements made on Wednesdays. The difference in body weight between weekly weigh-ins was used to calculate a daily weight gain (Table 6 and 7, and Figures 3 and 4). Individual body weight gain data are found in Appendix C.

Similar to the body weight results for both the males and females, there was no statistical difference in daily body weight gain between the exposure groups and controls for any one week with the exception of study days 71, 78 and 85. The body weight gains on study day 71 for the males and females in the low concentration exposure group were significantly less than controls, relating to the overall body weight differences as discussed above. On days 78 and 85, body weight gain was significantly greater than controls for females and males in the low exposure group.

Table 6. Group Average Daily Weight Gain, Males

Study Day	Control			Low (200 mg/m^3)			Intermediate (700 mg/m^3)			High (2000 mg/m^3)	
1	3.8	±0.7		3.8	±0.3		3.3	±0.2		3.8	±0.6
8	2.9	±0.6		2.7	±0.7		3.3	±0.9		2.5	±0.3
15	2.2	±0.6		2.5	±0.3		1.9	±0.4		1.7	±0.4
22	1.8	±0.4		1.5	±0.4		1.8	±0.7		1.6	±0.6
29	2.0	±0.5		1.6	±0.5		1.9	±0.9		1.6	±0.4
36	1.9	±0.8		1.7	±0.3		1.6	±0.5		1.8	±0.5
43	1.5	±0.6		1.0	±0.6		1.0	±1.8		1.3	±0.4
50	1.0	±0.4		1.2	±0.6		1.3	±1.4		1.3	±0.5
57	1.4	±0.4		1.7	±0.3		1.8	±0.3		1.8	±0.5
64	1.1	±0.5		1.2	±0.3		1.1	±0.3		0.9	±0.2
71	1.1	±0.6		-1.6	±0.6	*	1.1	±0.3		0.9	±0.4
78	1.1	±0.7		2.3	±0.4	*	0.9	±0.5		1.0	±0.3
85	0.9	±0.6		1.7	±0.5	*	0.6	±0.4		1.0	±0.7
92	0.6	±0.6		0.8	±0.5		0.6	±0.8		0.5	±0.7
99	0.6	±0.5		0.3	±0.5		0.8	±0.6		0.6	±0.8
106	-0.9	±1.2		-0.8	±1.2		-0.7	±1.5		-1.0	±2.5
107	-2.3	±0.4		-2.4	±0.7		-2.6	±0.4		-2.7	±0.3

Notes: average ± standard deviation; *P < 0.05, statistical significance from control group

Table 7. Group Average Daily Weight Gain, Females

Study Day	Control			Low (200 mg/m^3)			Intermediate (700 mg/m^3)			High (2000 mg/m^3)	
1	2.1	±0.3		2.4	±0.7		2.2	±0.4		2.2	±0.3
8	1.3	±0.4		1.5	±0.6		1.4	±0.3		1.2	±0.4
15	1.0	±0.3		1.1	±0.3		1.0	±0.4		0.9	±0.4
22	0.7	±0.5		1.0	±0.5		0.8	±0.3		0.7	±0.3
29	0.8	±0.3		0.7	±0.3		0.6	±0.3		0.6	±0.2
36	0.5	±0.3		0.8	±0.7		0.8	±0.5		0.5	±0.4
43	0.6	±0.7		0.4	±0.5		0.5	±0.6		0.6	±0.2
50	0.6	±0.4		0.5	±0.4		0.4	±0.6		0.7	±0.4
57	0.4	±0.3		0.8	±0.4		0.8	±0.3		0.9	±0.5
64	0.4	±0.5		0.4	±0.2		0.2	±0.3		0.0	±0.6
71	0.3	±0.4		-0.9	±0.5	*	0.1	±0.4		0.3	±0.5
78	0.3	±0.5		0.8	±0.5	*	0.5	±0.3		0.1	±0.5
85	0.2	±0.3		0.8	±0.5	*	0.4	±0.4		0.0	±0.4
92	0.5	±0.3		0.1	±0.5		0.3	±0.3		0.5	±0.5
99	0.1	±0.4		0.4	±0.5		0.1	±0.2		0.2	±0.4
106	-0.6	±1.0		-0.8	±0.7		-0.4	±1.1		-0.7	±1.1
107	-1.3	±0.4		-1.5	±0.1		-1.7	±0.2		-1.3	±0.5

Notes: average ± standard deviation; *P < 0.05, statistical significance from control group

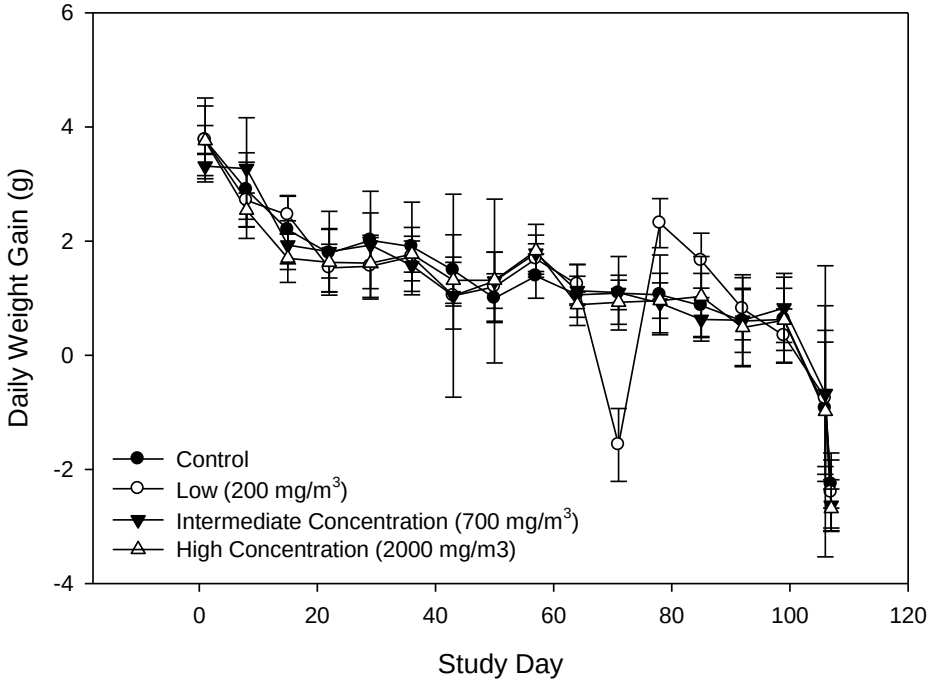

Figure 3. Group Average Body Weight Gain, Males. Shapes indicate weekly group average; bars indicate standard deviation.

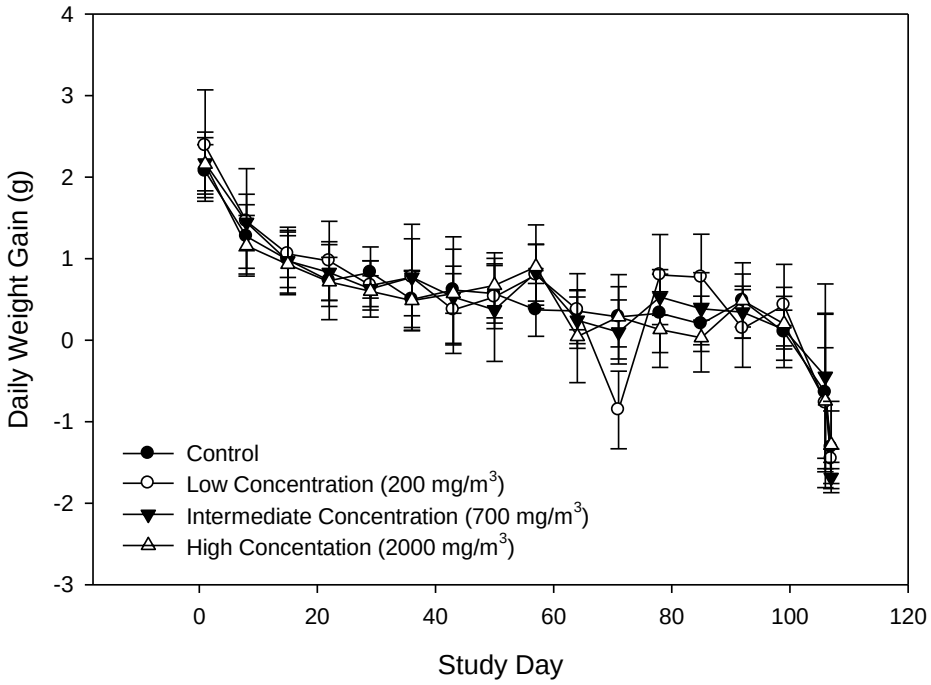

Figure 4. Group Average Body Weight Gain, Females. Shapes indicate weekly group average; bars indicate standard deviation.

4.6 Food Consumption

Food consumption was measured by weighing food trays on a regular basis and calculating a daily rate (Tables 8 and 9). Individual food consumption data are found in Appendix C. As with body weight measurements, there were no strong dose-related trends. There are only occasional statistically significant differences from control group food consumption that were likely not biologically significant.

The only noteworthy change in food consumption occurred in the low exposure concentration group. Consumption dropped significantly during week 11, which coincided with the decreased weight gain described in Section 4.5 above. The water line to the rack holding the caging for the low exposure group animals may have been restricted and the system was carefully checked to ensure that it was fully operational. Food consumption during the following week (week 12) in the low exposure group was higher than controls and the other exposure groups; consumption thereafter was equivalent among the groups.

Table 8. Food Consumption, Males

Group	Control		Low (200 mg/m^3)		Intermediate (700 mg/m^3)		High (2000 mg/m^3)	
Week 1	12.6	±2.2	13.2	±1.9	11.5	±1.3	10.0	±1.6 *
Week 2	16.9	±2.1	17.5	±1.0	16.5	±1.7	15.6	±1.2
Week 3	14.7	±1.9	15.2	±0.8	14.3	±1.4	13.9	±1.3
Week 4	16.9	±1.6	17.9	±1.1	17.0	±1.3	16.0	±1.6
Week 5	16.5	±1.8	15.6	±1.3	15.4	±1.5	14.7	±1.5
Week 6	17.3	±1.6	16.2	±1.2	15.7	±0.9	15.5	±1.4*
Week 7	16.5	±1.6	15.3	±1.5	15.3	±1.0	15.3	±1.5
Week 8	16.9	±1.4	17.6	±1.2	17.0	±0.9	17.0	±0.8
Week 9	16.6	±1.5	17.1	±1.2	17.2	±0.8	16.7	±1.3
Week 10	16.6	±1.6	17.0	±1.3	17.1	±0.9	16.8	±1.0
Week 11	16.8	±1.6	13.8	±1.0*	16.7	±0.6	16.3	±1.2
Week 12	16.6	±1.5	18.4	±0.7*	16.9	±0.7	17.0	±1.2
Week 13	17.3	±1.4	18.8	±1.0	17.7	±1.2	17.7	±1.2
Week 14	17.8	±1.2	18.6	±1.1	18.4	±1.4	18.1	±1.3
Week 15	18.8	±2.5	19.8	±2.3	19.6	±2.4	19.1	±1.4
Week 16	17.1	±1.4	17.8	±1.8	18.3	±1.2	14.8	±4.2

Notes: daily average food consumption (g) ± standard deviation; *P < 0.05, statistical significance from control group

Table 9. Food Consumption, Females

Group	Control		Low (200 mg/m^3)		Intermediate (700 mg/m^3)		High (2000 mg/m^3)	
Week 1	7.4	±1.8	8.5	±1.2	7.0	±1.6	6.9	±2.6
Week 2	11.8	±0.8	13.8	±4.7	11.6	±1.0	11.2	±1.4
Week 3	10.0	±1.0	10.3	±0.8	10.4	±1.2	9.6	±0.8
Week 4	12.4	±1.1	13.3	±1.0	12.6	±0.9	12.0	±0.8
Week 5	11.4	±1.1	11.2	±1.1	11.2	±1.1	10.4	±0.5
Week 6	12.1	±1.0	11.5	±1.4	11.4	±1.0	11.0	±0.8
Week 7	10.9	±0.9	10.1	±1.0	10.5	±0.8	10.4	±0.8
Week 8	11.5	±1.2	12.2	±0.6	12.2	±0.5	12.1	±0.6
Week 9	11.1	±0.9	11.4	±0.8	12.3	±0.6*	11.8	±0.6
Week 10	11.1	±0.7	11.4	±0.6	11.9	±0.6	11.2	±1.1
Week 11	11.1	±0.6	9.2	±1.1*	11.6	±0.6	11.0	±1.0
Week 12	10.7	±1.1	12.0	±1.6*	12.0	±0.8*	11.6	±0.8
Week 13	11.4	±0.8	12.3	±1.3	12.4	±0.8	11.9	±1.1
Week 14	12.2	±1.7	11.9	±2.3	13.2	±0.6*	13.5	±2.9
Week 15	12.2	±1.9	13.0	±0.7	13.2	±1.2	13.1	±1.3
Week 16	11.5	±2.0	11.6	±0.8	12.4	±1.3	15.6	±6.8*

Note: daily average food consumption (g) ± standard deviation

4.7 Clinical Observations

Two accidental deaths occurred unrelated to test compound administration; tissues were not evaluated microscopically. Additionally, several unrelated events were recorded in Appendix C, which include: animal 13 had a reddened left eye on study day13 that resolved the next day; on study day 62, animal 51 had a reddish discharge around the nose; on study day 64, animal 66 appeared to have a head tilt to the left; and on study day 93, animal 64 had sores or cuts at the end of its tail. In each of the listed cases, there were no further observations on subsequent days; hence these were considered to be isolated, unique observations that were unrelated to the test compound administration. Further minor observations (incidence of facial crust) found during the FOB procedure are presented in Section 4.11.2.

4.8 Ophthalmological Exams

Ophthalmologic examinations were conducted prior to the start of exposures and near the end of the exposure regimen. At the initial exam, animal 66 was found to have a hemorrhage in the anterior chamber of the right eye, with a pupil that failed to dilate. This finding was not considered to be of sufficient severity to warrant excluding the rat from the study. However animal 66 was reassigned to the control group and in exchange animal 8 was put into the high concentration group to avoid association of the hemorrhage with HEFA-C fuel. Also observed was chromodacryorrhea, which is a red-colored lacrimal secretion from the Harderian gland, which may be associated with general stress to the animal. In the pre-study ophthalmological exam, chromodacryorrhea was observed in one animal.

The ophthalmological exam conducted at the end of the exposure regimen found that chromodacryorrhea was observed in many animals, with numbers relatively evenly distributed across exposure groups (with 6/20, 8/20, 6/19 and 3/19 animals affected in the control, low, intermediate and high concentration exposure groups respectively. The hemorrhage observed in animal 66 at the beginning of the study was observed at the end of the study, also. There were no other significant findings in the ophthalmologic exam.

4.9 Sperm Motility

Sperm motility results were evaluated three ways: motility measurements from all samples, samples from the cauda only, and samples from the caput only. The results were analyzed for differences from control. For all three cases, the differences in mean values among the exposure groups were not great enough to be statistically different from controls. Sperm concentration measurements from the right testicle and right epididymis showed no statistically significant difference of any of the exposure groups from control. The exposures were not associated with any observable differences in motility or concentration. A complete report of the sperm motility observations can be found in Appendix E.

4.10 Vaginal Cytology

The vaginal lavage and cytology identified the predominant cell type present each day over a five-day span (Table 1). The cell types identified represented the proestrus (early and late), estrus and diestrus stages of the estrous cycle. All of the females showed the presence of cells representing at least two of the four stages. All of the exposed female rats appeared to be going through the estrus cycle, regardless of exposure to the HEFA-C jet fuel at any concentration. A complete report on vaginal cytology can be found in Appendix F.

4.11 Neurotoxicity Results

All exposure groups were evaluated for neurobehavioral effects using a small battery of neurobehavioral tests including a motor activity assessment and FOB. The complete motor activity and FOB report can be found in Appendix D.

4.11.1 Motor Activity. No significant differences between exposure groups were detected for the males or females for any of the motor activity measurements. Analyses for total distance, activity time, average speed, total rears, percentage of time in center vs. perimeter, and total activity habituation (Figures 5 and 6) over 60 minutes showed no differences between exposure groups.

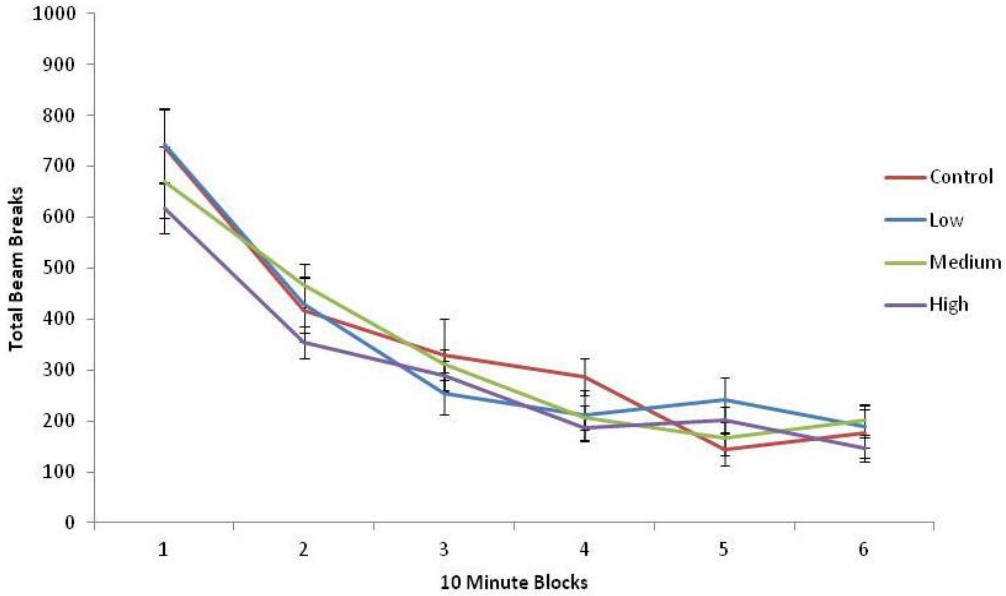

Figure 5. Motor Activity, Total Activity Habituation, Males. Colored lines indicate group average; bars indicate standard deviation.

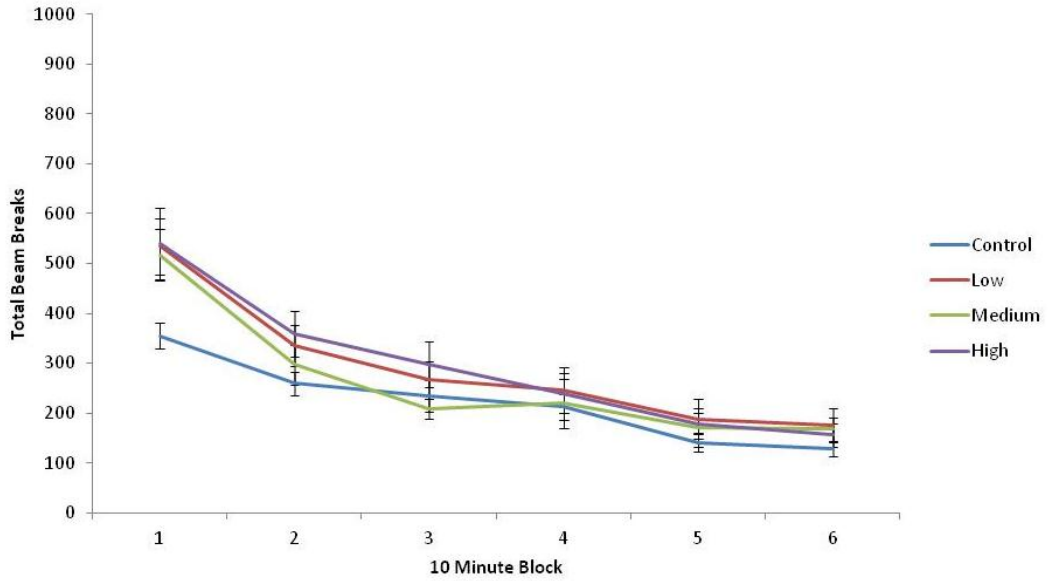

Figure 6. Motor Activity, Total Activity Habituation, Females. Colored lines indicate group average; bars indicate standard deviation.

4.11.2 FOB. For all functional observations including cage side, open field (e.g., number of rears) and manipulation tests (e.g., hind limb splay, forelimb grip), no dose related effects were reported for either males or females. Among observations during the FOB procedure, facial crust, on one eye, on both eyes, on the nose, or on multiple locations was noted for several males and females (Figures 7 and 8). Facial crust was noted around the nose in some males (2/10) in the control group, around one eye (1/10) in the low exposure concentration group, around one eye or the nose (3/10) in the intermediate concentration group, and around the nose (2/10) in the high exposure concentration group. In females, during the conduct of the FOB, facial crust was observed around one or both eyes, or in multiple locations (3/10) in the control group; around one eye or in multiple locations (4/10) in the low concentration group, one or both eyes (3/10) in the intermediate concentration group, and around the nose or multiple locations (3/10) in the high concentration group. As the observance of facial crust was distributed amongst the different exposure groups, there was no dose-related response; hence the appearance of facial crust could not be attributed to exposure to the HEFA-C test compound.

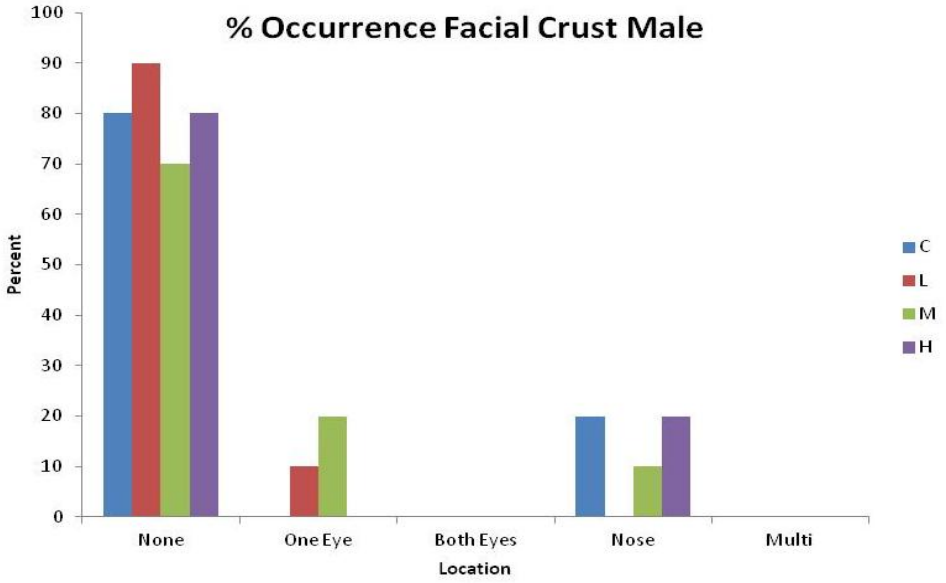

Figure 7: FOB Male Facial Crust Occurrence. Percent occurrence for control (C), low (L), medium (M) and high (H) exposure concentrations.

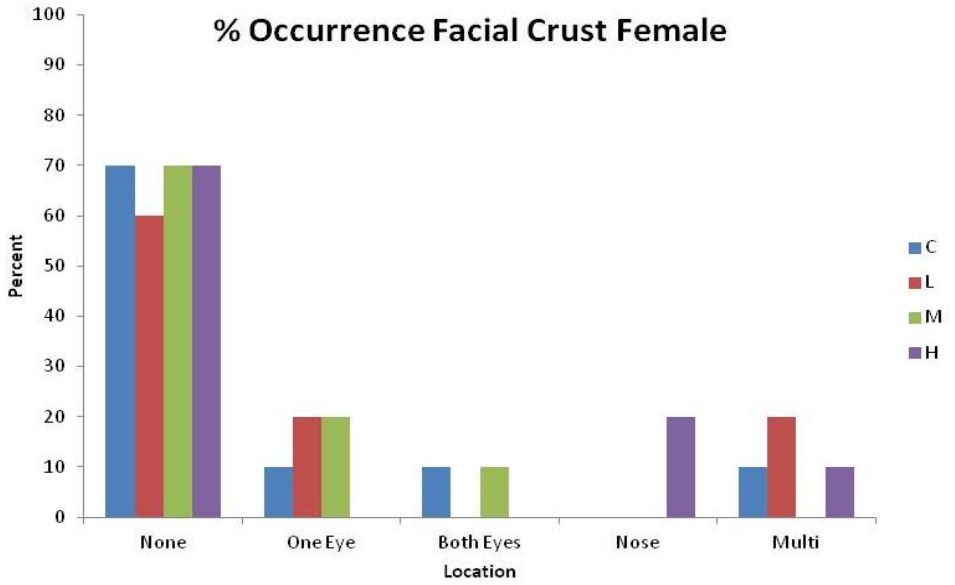

Figure 8: FOB Female Facial Crust Occurrence. Percent occurrence for control (C), low (L), medium (M) and high (H) exposure concentrations.

4.12 Gross Pathology

During necropsy, there was one gross observation and one incident of a missing tissue sample. A hydroureter was found in one kidney in one female (animal 62) exposed at the high exposure concentration. In one male (animal 77), only one adrenal gland was available for histopathology and may have been lost during necropsy. A table of gross pathology observations is located in Appendix C.

4.13 Organ Weights

Organs were weighed at necropsy. The average weight and standard deviation by exposure group were compiled for each organ system (Tables 10 and 11). Organ weight to body weight and organ weight to brain weight ratios were calculated and also presented in Tables 10 and 11. Individual data tables are located in Appendix C.

For the male rats, the organ weights did not show any significant difference for the exposed groups versus control. When the organ weight to body weight ratios were calculated (Table 10), the male rat right kidney weights in the intermediate group and both the right and left kidney weight ratios in males in the high dose group were significantly larger than controls. This may reflect a slight effect of accumulation or enlargement of hyaline droplets in male kidneys as reflected in the α_{2u}-globulin assay (Section 4.16), though a corresponding pathology observation

was not seen. The organ weight to brain weight ratio data did not indicate any statistically significant differences between exposure groups and controls.

For females (Table 11), the only organs that showed a significant weight difference from controls were spleen and liver, both in the intermediate exposure group. As the high exposure group did not show a difference from controls or from the intermediate exposure group, this effect did not appear to be correlated to exposure. To account for potential body weight differences, organ to body weight ratios were calculated. Spleen to body weight ratio in the intermediate concentration group was slightly elevated compared with the control ratio. In addition, the liver to body weight ratio was also slightly elevated in the intermediate and high concentration groups. Finally, both the right and left kidney in the high concentration exposure groups had a small but statistically significant increase in organ to body weight mass compared with controls. Among organ to brain weight ratios, the only statistically significant difference from control values was found in the liver to brain weight ratio from the intermediate exposure concentration group. As a strong dose-related trend was not seen, the biological significance of the spleen or liver weight increase in the intermediate concentration exposure females is not known.

Table 10. Organ Average Weights at Necropsy, Males

		Group 1 Control	Group 2 Low	Group 3 Intermediate	Group 4 High
Body Weight (g)	Average	330.0	328.7	320.9	308.6
	SD	30.1	11.9	12.9	19.0
	Ratio to Brain	173.7	174.2	170.1	164.5
Spleen (g)	Average	0.668	0.657	0.676	0.655
	SD	0.052	0.045	0.033	0.054
	% BW	0.203	0.200	0.211	0.212
	Ratio to Brain	0.352	0.348	0.358	0.350
Heart(g)	Average	0.892	0.903	0.901	0.868
	SD	0.079	0.050	0.082	0.065
	% BW	0.271	0.275	0.280	0.282
	Ratio to Brain	0.470	0.479	0.477	0.464
Thymus (g)	Average	0.204	0.188	0.195	0.177
	SD	0.024	0.016	0.026	0.032
	% BW	0.062	0.057	0.061	0.057
	Ratio to Brain	0.107	0.100	0.103	0.094
Brain (g)	Average	1.899	1.889	1.889	1.884
	SD	0.079	0.089	0.077	0.120
	% BW	0.578	0.575	0.589	0.613
	Ratio to Brain				
Right Kidney (g)	Average	1.018	1.040	1.046	1.026
	SD	0.060	0.057	0.047	0.067
	% BW	0.309	0.316	0.326*	0.333*
	Ratio to Brain	0.536	0.551	0.554	0.548
Left Kidney (g)	Average	1.025	1.064	1.058	1.034
	SD	0.049	0.053	0.055	0.081
	% BW	0.312	0.324	0.330	0.335*
	Ratio to Brain	0.540	0.564	0.560	0.552
Adrenal Glands (g)	Average	0.051	0.050	0.052	0.048
	SD	0.006	0.004	0.008	0.009
	% BW	0.015	0.015	0.016	0.015
	Ratio to Brain	0.027	0.027	0.028	0.027
Liver (g)	Average	9.639	9.743	9.932	9.367
	SD	1.223	0.717	1.502	0.675
	% BW	2.914	2.963	3.086	3.035
	Ratio to Brain	5.069	5.156	5.250	4.994
Right Epididymis (g)	Average	0.482	0.476	0.484	0.471
	SD	0.033	0.024	0.029	0.025
	% BW	0.146	0.145	0.151	0.153
	Ratio to Brain	0.254	0.252	0.257	0.251
Right Testicle (g)	Average	1.598	1.531	1.544	1.540
	SD	0.114	0.080	0.070	0.055
	% BW	0.486	0.466	0.481	0.500
	Ratio to Brain	0.842	0.810	0.819	0.820

Table 10. Organ Average Weights at Necropsy, Males (continued)

Epididymis Minus Cauda (g)	Average	0.277	0.272	0.277	0.267
	SD	0.025	0.019	0.027	0.018
	% BW	0.084	0.083	0.086	0.087
	Ratio to Brain	0.146	0.144	0.146	0.142
Left Testicle w/Epididymis (g)	Average	2.154	2.058	2.074	2.017
	SD	0.126	0.169	0.095	0.099
	% BW	0.655	0.626	0.647	0.656
	Ratio to Brain	1.135	1.088	1.099	1.073
Left Testicle w/out Epididymis (g)	Average	1.659	1.573	1.585	1.539
	SD	0.104	0.130	0.069	0.093
	% BW	0.5043	0.4785	0.4943	0.5003
	Ratio to Brain	0.874	0.832	0.840	0.818
Left Epididymis (g)	Average	0.500	0.478	0.492	0.480
	SD	0.036	0.043	0.029	0.021
	% BW	0.152	0.146	0.153	0.156
	Ratio to Brain	0.263	0.254	0.262	0.257

Note: * $P < 0.05$, statistical significance from control group

Table 11. Organ Average Weights at Necropsy, Females

		Group 1 Control	Group 2 Low	Group 3 Intermediate	Group 4 High
Body Weight (g)	Average	176.2	182.3	181.1	170.8
	SD	11.3	7.4	8.2	8.4
	% BW	100	100	100	100
	Ratio to Brain	100.1	103.9	102.7	99.0
Spleen (g)	Average	0.447	0.469	0.497*	0.439
	SD	0.042	0.023	0.028	0.039
	% BW	0.254	0.258	0.275*	0.257
	Ratio to Brain	0.254	0.268	0.282	0.254
Heart(g)	Average	0.594	0.658	0.668*	0.607
	SD	0.048	0.110	0.063	0.050
	% BW	0.337	0.361	0.369	0.356
	Ratio to Brain	0.337	0.374	0.379	0.352
Thymus (g)	Average	0.174	0.184	0.180	0.165
	SD	0.016	0.019	0.019	0.027
	% BW	0.099	0.101	0.099	0.097
	Ratio to Brain	0.099	0.105	0.102	0.096
Brain (g)	Average	1.761	1.757	1.767	1.727
	SD	0.051	0.068	0.080	0.086
	% BW	1.003	0.965	0.977	1.012
	Ratio to Brain				
Right Kidney (g)	Average	0.594	0.627	0.630	0.624
	SD	0.037	0.033	0.028	0.049
	% BW	0.337	0.344	0.348	0.365 *
	Ratio to Brain	0.337	0.358	0.357	0.361
Left Kidney (g)	Average	0.609	0.638	0.656	0.633
	SD	0.045	0.030	0.042	0.033
	% BW	0.345	0.350	0.362	0.371 *
	Ratio to Brain	0.346	0.364	0.372	0.367
Adrenal Glands (g)	Average	0.056	0.055	0.057	0.059
	SD	0.003	0.003	0.004	0.006
	% BW	0.032	0.030	0.031	0.035
	Ratio to Brain	0.032	0.031	0.032	0.034
Liver (g)	Average	4.656	4.901	5.164*	4.838
	SD	0.354	0.347	0.171	0.327
	% BW	2.645	2.689	2.854*	2.832 *
	Ratio to Brain	2.645	2.794	2.929*	2.804

Table 11. Organ Average Weights at Necropsy, Females (continued)

Uterus and Ovaries (g)	Average	0.863	0.778	0.773	0.727
	SD	0.328	0.240	0.214	0.315
	% BW	0.495	0.428	0.426	0.426
	Ratio to Brain	0.492	0.445	0.438	0.423
Uterus (g)	Average	0.762	0.680	0.673	0.636
	SD	0.329	0.237	0.218	0.307
	% BW	0.438	0.374	0.371	0.373
	Ratio to Brain	0.435	0.389	0.380	0.371
Ovaries (g)	Average	0.101	0.099	0.100	0.090
	SD	0.010	0.011	0.014	0.018
	% BW	0.057	0.054	0.055	0.053
	Ratio to Brain	0.057	0.056	0.057	0.052

Note: * $P < 0.05$, statistical significance from control group

4.14 Pathology

Tissues were collected at necropsy and preserved in formalin, except for testes, which were preserved in Bouin's solution. Tissues were shipped to an outside contractor for histopathology. The outside laboratory received the tissues, processed, embedded, cut the tissues and stained them for microscopic examination. A pathologist (Michelle W. Elliott, DVM, PhD, DACVP) conducted the pathology read; the full pathology report can be found in Appendix G.

There were minor findings (e.g., fibrosis or mononuclear infiltrate in the heart and mineralization in the trachea) that were of a nature commonly observed in this strain and age of rats and of a similar incidence and severity in control and treated rats, and so were considered incidental and unrelated to the administration of HEFA-C fuel.

One observation was chronic progressive nephropathy in kidneys of male rats only. Minimal focal and multifocal nephropathy was observed in all exposure groups. One mild case was observed in a male rat exposed at 2000 mg/m^3.

Hyaline drops were also observed in the kidney cells in all exposure groups. The kidney findings are not uncommon in adult male rats, as these rats begin to produce the protein alpha 2-microglobulin in the liver at puberty. As the males age, the protein tends to accumulate and is observed as hyaline droplets in the kidney cells. Some compounds affect the rate of accumulation, and the hyaline droplet size in cells may be observed to increase in size with increasing treatment concentration.

The observations that were considered to be related to HEFA-C fuel inhalation included areas of goblet (mucus) cell hyperplasia and degeneration of the olfactory epithelium. These observations were seen only in the animals exposed at the highest concentration of HEFA-C fuel.

4.15 Clinical Chemistry and Hematology

Clotting effectiveness as expressed by the prothrombin time did not significantly differ for either male or female rats in any of the exposure groups compared with the control animals. There were no significant differences in hematological parameters (white blood cells, erythrocytes, etc.) between control and exposed animals at any concentration for male or female rats.

For clinical chemistry analytes in male rats, there was an elevated albumin and total protein in the low concentration animals (3.64 mg/mL) as compared with controls (3.18 mg/mL), but not the higher concentration animals. In addition, the total bilirubin was also elevated in the male rats exposed at the lower concentration, but not at the intermediate or high concentration groups. None of the other clinical chemistry parameters were significantly different in the exposed animals compared with controls.

While decreased levels of total protein or albumin can be associated with liver dysfunction, reasons for elevated levels of albumin and total protein found in this study are not apparent. Since albumin constitutes a significant portion of total protein, the elevated total protein results from the elevated albumin, a correlation which can be seen in the individual animal data (data not shown). Increased albumin is rare, but may be associated with dehydration. Other factors associated with dehydration such as increased concentrations of electrolytes (Na^+, K^+ or Cl^-) are not seen. Liver enzymes (AST, ALP and ALT) were not significantly different from controls, so there is no other evidence for liver injury. Increased total bilirubin may be a sign of increased hemolysis or the incapacity of the liver to remove bilirubin, but there is no other evidence for liver damage as the liver enzymes in the exposed groups are not significantly different from the control group.

In female rats, the only clinical chemistry parameter that was significantly different was globulin, which was slightly decreased in rats exposed at the low concentration. Upon inspection of the individual animal data, there was one female rat (animal 32) in which the globulin value was much lower (1.2 g/dL) compared with the other animals in the group (between 2.1 and 2.3 g/dL). Also in that animal, albumin was elevated (4.5 versus 2.2 to 3.3 g/dL for the other females in the group). An increased albumin to globulin ratio may be related to an immune disorder characterized by a reduction in globulins. As only one animal exhibited the decreased globulin and increased albumin, with no other clinical chemistry effects, and no dose response with the other exposure groups, the biological significance of the response was not considered to be related to the animal exposure to HEFA-C. See Appendix H for further information regarding analytes and results of the clinical chemistry and hematology.

4.16 Alpha 2-Microglobulin

The protein alpha 2-microglobulin was measured in kidney samples for both female and male rats. See Appendix I for further information regarding methods and results. Levels in female kidney samples were approximately two orders of magnitude lower than in males. The amounts

found in the female exposed groups were not significantly different from the female control group. The level of alpha 2-microglobulin found in male rat kidneys was elevated compared with controls (54.8 µg/mg total protein), with the concentrations in the intermediate (80.5 µg/mg) and high (80.9 µg/mg) exposure concentration group animals significantly higher than in control animals. The low (75.2 µg/mg) exposure concentration group had slightly elevated levels of alpha 2-microglobulin compared with controls, though it was not statistically significant.

4.17 Micronuclei Assessment

Following a two-week exposure to HEFA-C fuel, rats showed no significant difference in the percentage of reticulocytes compared with air-exposed controls. The exposed animals did show a slight difference in the percentage of micronucleated reticulocytes; however, this difference was not dose-related. Micronucleated reticulocytes in low and high exposure groups were similar, while the percentage in the intermediate exposure group was lower than controls (Figures 9 and 10). See Appendix J for the full micronucleus report.

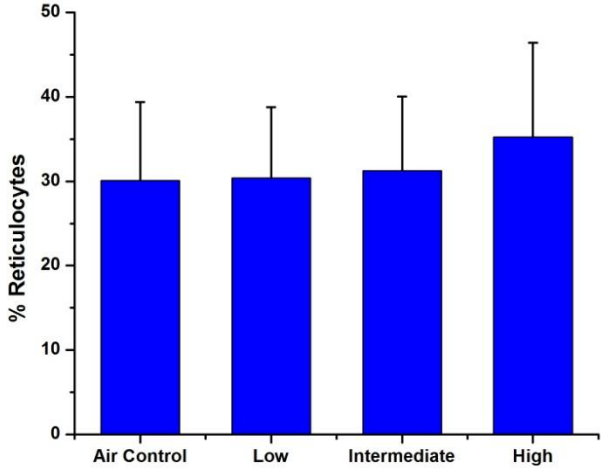

Figure 9: Percent Occurrence of Reticulocytes in Micronucleus Assay. Percentages compare for air control, low, medium and high exposure concentrations.

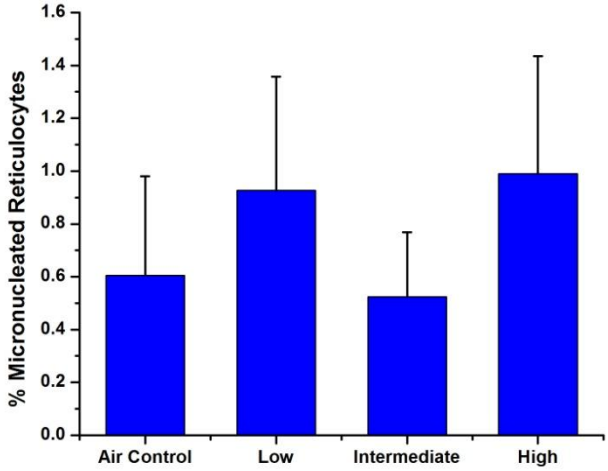

Figure 10: Percent Occurrence of Micronucleated Reticulocytes in Micronucleus Assay. Percentages compare for air control, low, medium and high exposure concentrations.

5.0 DISCUSSION

Male and female F-344 rats were exposed by inhalation to an aerosol and vapor combination of HEFA-C fuel. Animals were exposed to three target concentrations, 200, 700 and 2000 mg/m^3, or a control exposure of clean air. Exposures were conducted for six hours/day, five days/week. Each rat incurred a total of 71 exposure days. The average analytical total concentrations were 0.9 ± 2.4, 194.8 ± 15.3, 719.8 ± 154.2 and 1973.2 ± 156.4 mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 exposure groups, respectively. The measured concentration in the control chamber was not zero due to multiple factors: electrical variability and noise in the measuring instrument near the zero value; operator error in re-zeroing the instrument at the start of a day's study; and the detection of compounds emitted by the animals. The average measured concentration was less than 0.5 percent of the low concentration, and considered to be insignificant.

For the intermediate and high dose animals, the mean exposure concentration is not the same as the average chamber concentration. As noted in Section 4.3, these animals were inadvertently switched between the intermediate and high concentration chambers on exposure day 48. The intermediate group was exposed to the high concentration (2000 mg/m^3) for one day, and the high concentration group was exposed to the intermediate concentration (700 mg/m^3) for one day. The control and low exposure group were unaffected. After that one-day switch, animals were placed in the proper chambers for the remaining exposures. The effect of one day's switch in exposure concentration on the overall average exposure concentration was minimal (less than 2.8% or 1.3% difference from target). And, as the switch took place in the middle of the exposure schedule, the animals had several weeks of exposure to the appropriate concentrations before any biological assessments were made. Thus, the switch of intermediate and high group animals for one day was considered to have no impact on the results of the study.

The average aerosol concentrations were 0.0 ± 0.0 (background), 0.0 ± 0.0, 4.6 ± 3.0 (0.7 percent) and 242.7 ± 34.6 (12 percent) mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 exposure groups, respectively. By comparison, the average aerosol concentrations for a study with HEFA from a mixed animal fats and oils feedstock (HEFA-F) were 0.12 ± 0.11 (background), 14.3 ± 3.3 (7 percent), 147.7 ± 11.5 (22 percent) and 551.7 ± 97.7 (28 percent) mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 exposure groups, respectively, in a two-week inhalation study (Mattie *et al.*, 2012).

The difference in aerosol percentages between the two HEFA inhalation studies implies either a fairly large difference in the chemical constituents due to the feedstock of each fuel, or a significant difference in the fuel generation systems. Each of the HEFA studies was conducted in a different laboratory; the current study was performed at NAMRU-Dayton (Wright-Patterson AFB OH), while the two-week study was conducted at The Hamner Institutes for Health Sciences (Research Triangle Park NC). In another inhalation study conducted at The Hamner, aerosol concentrations were also relatively high, especially at the highest concentration. Aerosol concentrations were 0.6, 12 and 33 percent aerosol, respectively, in a 90-day study of male and female rats exposed to 200, 700 and 2000 mg/m^3 SPK alternative jet fuel (Mattie *et al.*, 2011a).

In the NAMRU-Dayton facility, a study of Jet A with two separate two-week phases was previously performed, in which two different groups of rats were exposed to 500, 1000 and 2000 mg/m^3. The Jet A tested was a blend of fuel from five petroleum companies and is JP-8 if military additives are included. Aerosol percentages were 4.4, 5.7 and 12 for the first two week phase and 6.1, 10.4 and 18.9 for the second two week phase (Sweeney *et al.*, 2013). As the highest target concentrations in the HEFA-C and Jet A studies were the same (2000 mg/m^3), it is appropriate to compare the aerosol percent from these groups. In all of these NAMRU-Dayton studies, the percentages for the high dose were similar (12, 12 and 18.9 percent). It appears that the aerosol concentrations measured in a particular study may be closely tied to the laboratory atmosphere generation system.

Animals exposed to the high concentration of HEFA-C fuel showed somewhat lower body weights as compared to control animals. In the high concentration (2000 mg/m^3) group, the average male body weight was decreased by approximately 5 percent, while the average female body weight decreased by 3 percent by the end of the exposures. Animals exposed to the high concentration (2000 mg/m^3) of FT jet fuel also showed lower body weights; the average male body weight was decreased by approximately 12 percent, while the average female body weight decreased 5 percent by the end of the 90-day exposures, relative to the control average (Mattie *et al.*, 2011a). While there was an association between decreased food consumption and SPK exposure, there was no difference in food consumption with HEFA-C among the high exposure group animals. In a previous JP-8 study, male and female rats were exposed to 0, 500 or 1000 mg/m^3 JP-8 vapor, continuously, for 90 consecutive days (Mattie *et al.*, 1991). Male rats in both exposure groups failed to gain weight compared to control rats in a statistically significant dose response manner (4.9 and 8.1 percent lower than controls). Female rat weight gains were not affected. HEFA-C exposure for 90-days did not reduce body weights as much as SPK or JP-8.

There were no clinical observations that could be related to the administration of the test material. No ophthalmological effects attributable to exposure to the HEFA-C fuel test material

were found after 90-days of exposure. No significant differences were observed for the male reproductive endpoints of interest. The vaginal cytology results did not show a significant alteration of the estrus cycle.

No jet fuel in the kerosene range has been shown to be a reproductive toxicant. Male and female reproductive endpoints were also not different from controls in the SPK 90-day study (Mattie *et al.*, 2011a). JP-8 was not found to be a reproductive toxicant in two reproductive studies performed as part of a 90-day oral investigation of JP-8 in rats (Mattie *et al.*, 1995) and reported later (Mattie *et al.*, 2000). In the first study, male rats were given 0, 750, 1500 or 3000 mg/kg neat JP-8 daily by gavage for 70 days prior to mating with naïve females to assess fertility and sperm parameters. After 70 days of dosing, body weights in the 3000 mg/kg group were over 30 percent lower than control weights; however, there were no significant changes for pregnancy rate, gestation length or sperm parameters as compared to control values. In the second reproductive study, general toxicity, fertility and reproductive endpoints were assessed in female rats dosed with neat JP-8 (0, 325, 750 or 1500 mg/kg) daily by gavage for a total of 21 weeks (90-days plus mating with naïve males, gestation and lactation). Results of the general toxicity endpoints revealed a significant dose-dependent decrease in body weights of the female rats. Significant organ weight ratio increases were seen for the liver:body, liver:brain and kidney:brain weights. Significant pathological changes were limited to squamous hyperplasia of the stomach and perianal dermatitis. Again, there were no statistically significant changes from control values for gestation length, pregnancy rate and numbers of pups per litter. There was a trend for decreased pup weight with increasing dose, likely related to maternal body weight decreases; pups from the 1500 mg/kg dosed females were statistically and biologically significantly lower in weight on postnatal days 4 through 21. Recovery occurred by 90 days. Based on the results of both reproductive studies, the no observed adverse effect level (NOAEL) for JP-8 reproductive and development effects is 750 mg/kg with 1500 mg/kg as a lowest observed adverse effect level (LOAEL) based on decreased pup weights (Mattie *et al.*, 2000).

Animals were assessed for neurobehavioral function via a FOB after the 13th week of exposure and motor activity after the 14th week. There were no significant observations relatable to exposure to HEFA-C fuel in either motor activity measurements or the FOB. By comparison, males rats exposed to the highest concentration of SPK jet fuel showed a reduction in total activity, and females in the same group responded with a reduction in initial exploratory activity. The only evidence of neurotoxicity seen in the FOB assessment of SPK rats was a reduction in rearing behavior observed in females exposed at the highest concentration (Mattie *et al.*, 2011a).

Multiple neurobehavioral studies have been undertaken to assess JP-8 effects. Neurobehavioral effects were assessed in adult rats following JP-8 vapor inhalation. Changes in behavioral response were observed in two studies where rats were exposed to 0, 500 or 1000 mg/m^3 for 6 hours/day 5 days a week for 6 weeks. When animals were subjected to different operant tasks with varying levels of complexity, the low and high exposure groups scored the same as control animals on all tests except for the most complex tasks. In these two operant tests, group differences emerged; low dose animals demonstrated better performance than high dose animals while neither group performed differently from controls (Ritchie *et al.*, 2001). In a second study using the same exposure methods, animals were tested in a large battery of neurobehavioral tasks. No exposure group differences were found in acoustic startle responses, forelimb grip

strength, nociception, social interaction, the forced swim test, spontaneous locomotor activity, passive avoidance or Morris water maze performance. However, differences were found in a test for behavioral sensitization. The appetitive stimulus approach sensitization assay measures the time an animal spends proximal to an appetitive stimulus versus a neutral stimulus. Animals exposed to JP-8 spent more time than control animals investigating the appetitive stimulus, suggesting behavioral sensitization and altered neural pathways related to the dopaminergic system (Rossi *et al.*, 2001). Overall, only two very specific neurobehavioral effects of JP-8 vapor were seen after exposure in adult rats.

Animals were necropsied the day after the last exposure and examined for gross lesions. There were no gross lesions relatable to HEFA-C exposure. This is consistent with SPK (Mattie *et al.*, 2011a) and JP-8 exposure (Mattie *et al.*, 1991, 1995).

Differences in the average organ weights were not significant except for a spleen and liver weight increase in the intermediate concentration exposure females. Since the weight increases did not demonstrate a dose-response relationship with exposure to HEFA-C fuel, they appear to be random biological variability. Organ weight differences seen at the high dose in the SPK study were correlated with body weight decreases and not considered to be a direct effect from the fuel (Mattie *et al.*, 2011a). JP-8 also did not produce changes in organ weights after exposure continuously for 90-days except in the male rat kidneys, where there was a significant increase in hyaline droplet formation (Mattie *et al.*, 1991).

Tissues examined histopathologically showed no adverse effects in any target tissues other than the nasal tissues. Olfactory epithelial degeneration and goblet hyperplasia were observed in the nasal turbinate airways of the high concentration (2000 mg/m^3) male and female rats. In the SPK study, olfactory epithelial degeneration was also observed in the nasal airways at the high concentration (2000 mg/m^3 SPK) in both male and female rats. However, for SPK exposure, respiratory epithelial hyperplasia was also observed in the nasal airways. In the lung, minimal to mild multifocal areas of inflammatory cell infiltration occurred in the high concentration groups in male and female rats. A lesser effect was also seen in the lungs of the intermediate exposure groups (Mattie *et al.*, 2011a). Histologically, there were no effects in the nasal cavities or lungs of rats exposed to JP-8 for 90-days continuously (Mattie *et al.*, 1991). However, the JP-8 was only in vapor form and the aerosol appears to be required to produce changes in nasal cavity tissues and lung epithelium. In the Sweeney *et al.* (2013) study, there were no changes reported in the nasal cavities or lungs after exposure to Jet A for two weeks. In this study, two weeks of exposure to a mixed vapor and aerosol of Jet A was probably not long enough to cause effects seen after HEFA-C and SPK 90-day exposures.

There were a limited number of statistical differences in the clinical chemistry and hematology results for HEFA-C. These changes were not considered to be biologically significant due to high variability and lack of dose-response. No biologically significant changes were identified in clinical chemistry and hematologic analyses for male and female rats exposed to SPK in the 90 day study (Mattie *et al.*, 2011a) or for JP-8 for 90 days continuously (Mattie *et al.*, 1991).

The level of alpha 2-microglobulin concentration increased slightly in low dose rats, increased statistically in the intermediate dose rats and then essentially remained the same for the high dose

rats. Histopathology of the kidneys did not reveal any significant difference in hyaline droplets in the male rats in any concentration group compared with the controls. Compared to JP-8 exposure (Mattie *et al.*, 1991), the alpha 2-microglobulin response to HEFA-C exposure was not very strong; HEFA-C is considered a weak inducer of alpha 2-microglobulin accumulation in the F-344 male rat. FT jet fuel was an even weaker inducer of alpha 2-microglobulin than HEFA-C (Mattie *et al.*, 2011a).

HEFA-C was shown to be non-genotoxic by the micronucleus assay. Male and female rats in each dose group showed no significant difference in the percentage of reticulocytes compared with air-exposed controls following a two-week exposure to HEFA-C fuel. Therefore, there was no apparent toxicity to the reticulocytes caused by exposure to HEFA-C fuel. Although there was a slight difference in the percentage of micronucleated reticulocytes in exposed animals, the difference was not dose-related. HEFA-C and HEFA from a tallow feedstock (rendered beef fat, HEFA-T) were also found to be non-mutagenic by means of the *Salmonella-Escherichia coli*/microsome plate incorporation assay (Ames test) (Mattie *et al.*, 2013). HEFA-F was found to be negative in the Ames test by Riccio et al. (2010); HEFA-F was referred to in this study as R-8 or renewable JP-8 (POSF log book number 5469). Negative results in the combination of mutagenicity and genotoxicity assays increases the evidence that exposure to the HEFA jet fuels will not result in carcinogenic outcomes.

SPK was also shown to be non-mutagenic by the Ames assay (Mattie *et al.*, 2011b; Riccio *et al.*, 2010). Similarly, SPK was also found to be non-genotoxic in a micronucleus assay conducted with a two-week rangefinder study in which male and female rats were exposed to 500, 1000 and 2000 mg/m^3 SPK fuel (Mattie *et al.*, 2011c). SPK was also negative in a chromosomal aberration assay in which human lymphocytes were exposed to the fuel *in vitro* (Mattie *et al.*, 2011b).

JP-8 has been found to be non-mutagenic and non-genotoxic in multiple studies. Brusick and Matheson (1978) demonstrated that JP-8 was not mutagenic using the Ames assay, which was a relatively new test at the time. Mice were treated with either a single or multiple applications of JP-8 and Jet A fuels in a dermal variation of the mammalian micronucleus test. Using several different dermal exposure regimens, no statistically significant increases in the incidence of reticulocytes or micronucleated reticulocytes was observed in the bone marrow and/or peripheral blood of mice treated with JP-8 or Jet-A when compared with those of untreated control animals (Vijayalaxmi *et al.*, 2006; Vijayalaxmi, 2011).

6.0 CONCLUSIONS

HEFA-C jet fuel is similar to JP-8 but contains no aromatic compounds, similar to the SPK alternative jet fuel. The primary target organ for HEFA-C jet fuel appears to be the nasal tissues. In the nasal cavities, the olfactory epithelial degeneration and goblet hyperplasia of nasal epithelium were seen at the 2000 mg/m^3 dose. Overall, toxicity was less than the current jet fuel, JP-8.

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APPENDIX A. 90-DAY STUDY PROTOCOL

16 March 2011

U.S. AIR FORCE SPONSORED ANIMAL RESEARCH PROPOSAL SIGNATURE COORDINATION SHEET

I. **NAME OF FACILITY:**

Naval Medical Research Unit (NAMRU) – Dayton, Wright-Patterson AFB, OH

II. **PROTOCOL NUMBER:** F-WA-2011-**0126**-A

III. **PROTOCOL TITLE:** 90-Day Inhalation Toxicity Study of HRJ Fuel in Rats (*Rattus norvegicus*) with Neurotoxicity Testing and Genotoxicity Assay

IV. **PRINCIPAL INVESTIGATOR:**

LCDR William Howard, Ph.D.
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William Howard (Printed Name) | (Signature) | 21MAR2011 (Date)

V. **SCIENTIFIC REVIEW:** This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

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DAVID R. MATTIE (Printed Name) | (Signature) | 18Mar11 (Date)

16 March 2011

VI. STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal and ensured that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

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VII. ATTENDING VETERINARIAN: In accordance with Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

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16 March 2011

PROTOCOL TITLE

90-Day Inhalation Toxicity Study of HRJ Fuel in Rats (*Rattus norvegicus*) with Neurotoxicity Testing and Genotoxicity Assay

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I. NON-TECHNICAL SYNOPSIS

The United States military is developing alternatives to petroleum-based jet fuels, including synthetic and biologically-produced hydrocarbon liquids such as the Hydrotreated Renewable Jet (HRJ) fuel. There may be occupational and environmental exposure to the HRJ fuel during refueling, maintenance, or operation of military aircraft.

Inhalation is one of the primary routes of exposure so it is very important to study effects on the lungs and body after repeatedly breathing a chemical mixture such as jet fuel. Preliminary analysis of the new fuel suggests that the components are similar to JP-8, the traditional military fuel, but the overall composition is significantly different; therefore, the health effects associated with exposure to the alternative fuel may also be significantly different than with JP-8. This study will investigate the inhalation toxicity of the HRJ fuel, Camelina, over the minimum period of time required by regulators to establish an occupational permissible exposure level for safe use of the fuel. The HRJ will be administered by whole-body inhalation exposure at three concentrations plus a control level (2000, 700, 200, and 0 mg/m^3) to Fischer 344 rats (10 rats per sex per dose) on a repeated basis (6 hours per day) for 5 days per week for 13 weeks. Prior to the end of exposures, animals will undergo functional observations to look for signs of toxicity to the nervous system that might affect behavior. Potential reproductive toxicity in females will be studied by looking for changes in the female estrous cycle (analogous to the menstrual cycle in human females). Cells representative of the various stages of the estrous cycle will be collected by a saline wash (lavage) of the vagina of female rats. Reproductive toxicity in male rats will be studied in freshly collected sperm at necropsy by looking for changes in sperm movement (motility) and abnormal sperm shape (morphology). At the end of exposures, the rats will be euthanized, and various tissues collected, processed, and examined by microscope to look for evidence of damage to tissues that can be related to the inhalation of HRJ fuel. Correlation of any tissue damage with exposure concentration will help establish safe exposure guidelines for use of this important new class of renewable jet fuel alternatives.

A second experiment will be conducted concurrently in which F344 rats are exposed to HRJ fuel at the same concentrations for a two-week period. Following exposures, animals will be euthanized and bone marrow extracted from the femur. The marrow cells will be examined for the formation of micronuclei, a marker of damage to a cell's genetic material (genotoxicity). Damage to the cell's genetic material (DNA in chromosomes) can cause mutations, which could lead to cancer. When cells divide, damaged chromosomes may leave remnants called micronuclei that can be observed and counted. Thus, the micronucleus assay is used to identify compounds that could be genotoxic, and eventually carcinogenic. This second study will establish whether or not the HRJ fuel is genotoxic.

This protocol will use 150 rats. The final disposition of all rats on this protocol will be euthanasia by deep anesthetization using intraperitoneal injection of sodium pentobarbital followed by exsanguination via transection of the abdominal aorta.

II. BACKGROUND

II.1. Background

The Office of the Secretary of Defense Assured Fuels Initiative is pursuing domestically produced alternative fuels for military use to decrease dependence on foreign oil sources. These fuels would potentially be used in military aircraft, ships, and ground vehicles. Fuels are among the most common sources of military occupational exposures. Dermal contact and inhalation are generally the primary routes of exposure. Preliminary analysis of the new fuels shows that many of the ingredients are the same as JP-8, the traditional military fuel, but the composition is still different in each fuel. Therefore, the health effects associated with exposure to each alternative fuel may also be significantly different than JP-8.

One of the alternative fuels currently produced in the United States is a Fischer-Tropsch (F-T) fuel made from natural gas. RHPB conducted a Toxicology Program for this Fischer-Tropsch (F-T) fuel that along with JP-8 will provide the baseline data for comparing all future alternative fuels. There are two biobased jet fuels that are undergoing development and certification for use by the military. These new biobased jet fuels need to be examined for their toxicity potential compared to the JP-8 and F-T jet fuels. One of the biofuels, hydro-treated renewable jet (HRJ) fuel is based on oils extracted from the camelina plant (Camelina sativa). The oil is treated by a process of hydrogenation/de-oxygenation of free fatty acids to convert vegetable triglycerides to a synthetic paraffinic kerosene suitable for use as a jet fuel.

A 90-day inhalation study of JP-8 jet fuel (Mattie, *et al.*, 1991) was conducted with necropsies of test subjects at interim time points, at the end of exposures, and at postexposure recovery time points. Target concentrations were 0, 500, and 1000 mg/m^3 of JP-8 vapor with continuous exposure (24 hr/day) over 90 days. The primary effects seen in this study were body weight depression in males, but not females. JP-8 inhalation also resulted in alterations in the male rat kidney characterized by hyaline droplet formation, granular casts in the outer medulla, and nephrosis. The hyaline droplet formation and granular casts resolved in the recovery animals, but the nephrosis did not. These observations in the kidney have been found in male rats exposed to other hydrocarbons. They are considered to be specific to the male rat and have not been seen in humans.

The US EPA Health Effects Test Guidelines OPPTS 870.3465, 90-Day Inhalation Toxicity, were used to design the inhalation studies of F-T jet fuel (Wong, *et al.*, 2010; Mattie, *et al.*, 2010) conducted previously. The 90-day F-T synthetic jet fuel study had target concentrations of 0, 200, 700, and 2000 mg/m^3. In the high concentration (2000 mg/m^3) group, the average male body weight was decreased by approximately 12%, while the average female body weight decreased by 5% by the end of the exposures. Olfactory epithelial degeneration and respiratory epithelial hyperplasia were observed in the nasal airways of the high concentration (2000 mg/m^3) male and female rat. In the lung, minimal to mild multifocal areas of inflammatory cell infiltration were observed in the high concentration groups in male and female rats. A lesser effect was also seen in the lungs of the intermediate exposure groups. Similar to the JP-8 study, hyaline droplets were seen in male rat kidneys exposed at the highest concentration of F-T jet fuel.

The HRJ fuel has a chemical composition more similar to F-T jet fuel than to JP-8, and may thus have a toxicology profile closer to F-T jet fuel. A study with repeated administration of a test

material spanning a 90-day period is called a subchronic study and may be used to identify adverse effects not detected in shorter-term acute studies, identify a no-observable adverse effects level (NOAEL), and provide information for regulatory agencies. The 90-day inhalation study design is a standard toxicity test that is used to provide data that can help establish occupational exposure limits. The US EPA OPPTS 870.3465 guidelines will be used for this 90-day inhalation study of HRJ fuel for comparison to JP-8 and F-T jet fuels.

II.2. Literature Search for Duplication

II.2.1. Databases Searched

Dialog was used to search the following databases: Agricola, Dissertation Abstracts Online, BIOSIS Previews, Inside Conferences, EMBASE, International Pharmaceutical Abstracts, IPA Toxicology, MEDLINE, ToxFile, BIOSIS Toxline, and FEDRIP. The Defense Technical Information Center (DTIC) Biomedical Research Database, Research Summaries and Technical Reports, IR & D were searched. Also searched other databases: Cambridge Scientific Abstracts and Engineering Village.

II.2.2. Number, Date, and Resources of Search

Search # 2011153, was completed 12/27/2010 by Carol Reed, MLIS of the D'Azzo Research Library, Det 1, AFRL/WSC.

II.2.3. Period of Search

All dates to present

II.2.4. Key Words of Search

Kerosene, Jet A, jet fuel, JP-8, HRJ, hydrorenewable jet, HRJ Camelina, HRJ plant oils, HRJ Tallow, HRJ Animal Fats and Oils, biofuel, biobased/bio-based, toxicity/toxicology, animal, rat, mouse/mice, micronucleus, inhalation, acute, two-week, 90-day, subchronic, 13-week, sensory irritation (in lungs so respiratory tract), RD50, Alarie (scientist who developed and validated the RD50 sensory irritation methodology).

II.2.5. Results of Search

The results of this literature search found no articles for toxicity studies with HRJ jet fuels or other biofuels, although related articles were found for JP-8. Therefore, duplication of research efforts proposed for this project was not identified.

III. OBJECTIVE / HYPOTHESIS

Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of HRJ fuel by inhalation is needed to assess the risk of replacing or augmenting JP-8 by HRJ fuel. HRJ fuel has the potential for both vapor and aerosol (fuel droplets) exposures. The objective of this study is to assess the potential inhalation toxicity of a test substance when administered via inhalation exposure to Fischer 344 rats on a repeated basis for 90 days (5 days per week over 13 weeks, 65 total exposures). The assessments will include clinical observations, gross pathology, clinical pathology, and histopathology. Towards the end of the study, neurotoxicological effects will be assessed using a Functional Observational Battery and Motor Activity tests performed on rats exposed to HRJ fuel. In addition to histopathology of reproductive organs, reproductive toxicity will be examined via sperm morphology and vaginal cytology. Data will be used to establish no effect levels and lowest effect levels that are required by regulatory processes to establish occupational permissible exposure levels (PEL). A secondary objective is to determine the genotoxicity of inhaled HRJ fuel by exposing rats for two weeks to HRJ fuel and quantifying the formation of micronuclei in bone marrow. The hypothesis is that HRJ (similar to F-T jet fuel) will have the same or lesser degree of inhalation toxicity compared to JP-8; mild effects on rats at the high dose exposure, minimal to no effects at the mid dose, and no effects at the low dose exposure.

IV. MILITARY RELEVANCE

The military is pursuing a number of alternative fuels aimed at increasing domestic fuel production. One of the alternative fuels currently produced in the United States is a Hydrotreated Renewable Jet (HRJ) fuel made from oil extracted from the camelina plant. This fuel will be used in Air Force aircraft and ground vehicles, as well as ships and tanks. Certification of the Air Force fleet to fly using HRJ fuel is in progress. Fuels are among the most common sources of military occupational exposures. Inhalation is one of the primary routes of exposure, so it is very important to study effects on the lungs and body. Preliminary analysis of the new fuel suggests that there are chemical components similar to JP-8, the traditional military fuel. However, the overall composition is significantly different; therefore, health effects associated with exposure to the alternative fuel may also be significantly different than JP-8. The Air Force Research Laboratory Applied Biotechnology Branch (AFRL/711 HPW/RHPB) has been asked by the Air Force Alternative Fuels Certification Office and the Air Force Research Laboratory Fuels Branch to determine the potential health effects of the alternative fuels under development and certification. The AFRL/711 HPW/RHPB and the Naval Medical Research Unit - Dayton (NAMRU-D) have designed a toxicity testing program for these alternative fuels. The program includes specific toxicity tests required to develop a health hazard assessment for the fuel. A 90-day inhalation toxicity study of HRJ fuel will be conducted to provide data to be used to establish occupational permissible exposure levels for DoD personnel.

IV.1. Funding Agency

U. S. Air Force, AFMC, Alternative Fuels Certification Office, ASC/WNN

V. MATERIALS AND METHODS

V.1. Experimental Design and General Procedures

There are two components to this study. Experiment 1 is a 90-day inhalation toxicity study of F344 rats to an aerosol and vapor mixture of HRJ fuel with neurotoxicity testing (see V.1.1. Experiment 1, below). Rats will be exposed 6 h/d, 5 d/wk, for approximately 13 weeks (minimum 65 exposure days) in whole-body chambers. Standard endpoints for a 90-day study include motor activity (MA) and functional observation battery (FOB)(to assess neurotoxicity), clinical chemistry and hematology of blood samples, and histopathology.

The purpose of this study is to provide toxicity data associated with repeated inhalation of the test substance over a subchronic period of time (90-days). The number of animals/sex/group, 10 per sex per exposure concentration, was the minimum number specified by the U.S. EPA OPPTS 870.3465 90-Day Inhalation Toxicity and OECD 413, Subchronic Inhalation Toxicity: 90-day Study guidelines to provide data to permit the determination of a no-observed effect level (NOEL) and toxic effects associated with repeated exposures over 90 days in order to develop a risk assessment for HRJ fuel. The 90-day inhalation study of FT jet fuel (Wong *et al.*, 2010; Mattie, *et al.*, 2010) used a similar study design with 10 rats per sex per dose group. The 90-day JP-8 inhalation study (Mattie, *et al.*, 1991) was a more complex design, with necropsies of 10 animals per sex per group at interim time points, a necropsy of 15 rats per sex per group at the end of the 90-day exposure period, and additional animals in post-exposure recovery groups.

Experiment 2 consists of a two-week inhalation exposure of F344 rats to an aerosol and vapor mixture of HRJ fuel (concurrent with Experiment 1) with a genotoxicity assay based on the US EPA Health Effects Test Guidelines, OPPTS 870.5395, “Mammalian Erythrocyte Micronucleus Test” (see V.1.2. Experiment 2, below). The guidelines specify that there should be at least 5 analyzable animals per sex per treatment group and control group. For this study, 5 males and 5 females per exposure concentration (total of 40 animals) will be exposed to HRJ fuel. There will be an additional 10 males and 10 females, not exposed to jet fuel, for use as positive and vehicle controls, for a total of 60 animals for the micronucleus assay. Additional animals (5 males and 5 females) will be ordered for this study for use in training and method development for the micronucleus assay. These animals will be euthanized and dissected to extract bone marrow from the femur. The bone marrow will be processed and analyzed by flow cytometry for micronuclei, an indicator of genotoxicity.

Summary of overall experimental plan:

TABLE 1:

Group	Number of Animals	
	Males	Females
Experiment 1	40	40
Experiment 2	30	30
Training	5	5
Total	75	75

V.1.1. Experiment 1 - 90-Day Inhalation Toxicity Study of HRJ Fuel in Rats (*Rattus norvegicus*) with Neurotoxicity Testing

Test Guideline: This 90-day study design is based on the U.S. Environmental Protection Agency (U.S. EPA) Harmonized Test Guideline developed by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 870.3465, “90-Day Inhalation Toxicity”. The neurotoxicity testing follows the U.S. EPA Health Effects Test Guideline OPPTS 870.6200 “Neurotoxicity Screening Battery”.

Test Animal Selection: The rat is used as a surrogate for humans in the detection of toxicity and is a species in which known toxicants have been detected. This rodent species is commonly used in the conduct of toxicity studies and is recommended by several health effects test guidelines including those noted above. Historical control data are also available with this strain of rat for comparative evaluation, if necessary.

Number of Animals: Fischer 344 Rats, 40 males and 40 females will be used on the 90-day inhalation study.

Route, Duration and Frequency of Administration: The test substance, HRJ jet fuel, will be administered as an aerosol/vapor combination. The inhalation route is one of the potential routes of human exposure to this test substance. The test subjects will be exposed for 6 hours per day, 5 days per week over a 13-week period to assess the hazards that might arise from repeated subchronic exposure to this material.

A staggered start of two replicates of animals will be required in order to complete the necropsy at the end of the study period. A total of 40 animals can be necropsied on a day, given the available personnel and facilities. In order to provide the same number of days of exposure, the total number of exposure animals will be divided into two replicates. The start of exposures for each replicate will be staggered by one day in order to stagger the necropsy days at the end of the study.

Exposure Levels: Animals will be exposed to three concentrations of jet fuel, high (2000 mg/m^3), intermediate (700 mg/m^3) and low (200 mg/m^3), with a control group exposed to clean air (0 mg/m^3). These concentrations were selected based on results of previous 90-day inhalation studies with the chemically similar JP-8 jet fuel and FT synthetic jet fuel (see II.1.Background).

TABLE 2: Study Summary

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control Replicate 1	0	5	5
Control Replicate 2		5	5
Low Replicate 1	200	5	5
Low Replicate 2		5	5
Intermediate Replicate 1	700	5	5
Intermediate Replicate 2		5	5
High Replicate 1	2000	5	5
High Replicate 2		5	5
Total		40	40

Observations: Animals will be observed before and after exposures for overt signs of toxicity. During necropsy, the tissues and organs will be examined for gross pathology. At necropsy, blood will be collected for clinical pathology measurements. Tissues will be collected for histopathology, including nasal airways, trachea, larynx, lungs, liver, kidney, spleen, adrenals, heart, and others. Tissues in the control and high concentration group will be examined microscopically. If there are histopathological observations related to the exposure to the test substance, tissues from lower concentration groups may also be examined.

Motor Activity and Functional Observational Battery. A measure of motor activity (MA) and a functional observational battery (FOB) will be used to assess the neurotoxic effects of exposure to HRJ jet fuel vapor and aerosol.

Sperm Morphology and Vaginal Cytology Examinations. In order to assess the potential reproductive toxicity of inhaled HRJ jet fuel, sperm morphology and vaginal cytology examinations will be performed. Sperm from males at necropsy will be collected, stained, and examined for percentage of abnormal sperm. Alterations in the estrous cycle of females will be assessed by examination of vaginal cytology from vaginal smears collected daily over a week-long period.

TABLE 3: Study Timeline

	Sun	Mon	Tue	Wed	Thu	Fri	Sat
			Receive Animals Begin Quaran-tine				
		Begin Cage acclimation					
Week 1		Exposure (Exp)	Exp	Exp	Exp	Exp	Food consumption
Week 2-12	Food consumption	Exp	Exp	Exp	Exp	Exp	Food consumption
Week 13	Food consumption	Exp	Exp	Last Exp Replicate 1	Last Exp Replicate 2 Necropsy Replicate 1	Necropsy Replicate 2	

* In case of holidays

Quarantine and Acclimation Period: Shortly after their arrival at the laboratory, the animals will be transported to a room selected for the study for quarantine and acclimation. The animals will be removed from the shipping cartons and examined. All animals with evidence of disease or physical abnormalities will be euthanized and necropsied. If an unusually large number of animals show evidence of disease or physical abnormalities, the entire shipment of animals will be rejected for use in the study. Animals will be quarantined in the facility for 7 to 10 days. During the quarantine and facility acclimation period, animals will be individually housed in solid bottom plastic cages. Towards the end of quarantine during the week prior to start of exposures, rats will be acclimated to the stainless steel wiremesh cages (Toxic Hazard Research Unit or R-24 cage units or equivalent) in the animal room. Animals will be placed in the inhalation wire mesh cages for an increasing length of time, e.g., 1 hr, 2 hr, 3 hr, 4 hr, and then 6 hr on successive days. Prior to assignment to study, all animals will be examined by an animal care staff member to ascertain suitability for study.

Selection for Study: Animals considered suitable for study on the basis of pretest physical examinations, body weight data, and any other pretest evaluations such as the ophthalmologic exam, will be selected for this study. Body weights will be measured the day after arrival and prior to randomization for group assignment before beginning test substance exposure. During the acclimation period, animals will be randomly assigned to the groups in an attempt to equalize mean group body weights. Individual weights of animals placed on test will be within ± 20% of the mean weight for each sex. Disposition of all animals not utilized in the study will be maintained in the study records.

Test Substance Administration:

TABLE 4: Test Substance

Name:	**Hydrotreated Renewable Jet (HRJ) Fuel with additives: HRJ Camelina**
	Bio-oil Derived SPK
CAS #:	NA
Formula:	C_9-C_{15} paraffin bio-oil derived
Molecular Weight	NA, mixture
Description:	Colorless liquid
Test Substance Category:	Fuels. Jet Aircraft. OSHA combustible liquid.
Storage:	Keep container closed tightly in cool, well-ventilated place. Keep away from heat, and sources of ignition.
Stability:	Stable at normal conditions. Decomposes upon heating.
Supplier:	UOP through AFRL Fuels Branch
Lot Number:	POSF 6152

Expiration date: One year from date of receipt.

Route of Administration: The test substance, HRJ fuel, will be administered as an aerosol/vapor combination by inhalation in whole-body exposure as this is a major potential route of human exposure to this test substance.

Frequency and Duration of Administration: The test substance will be administered for 6 hours per exposure day, 5 days per week for a total of 65 exposures. The number of consecutive non-exposure days will not exceed two with exceptions for facility holidays, FOB assessments, severe weather, or other events which may preclude safe operation of the exposures. There will be at least 65 exposure days. To accommodate the numbers of animals in the FOB tests and at the terminal necropsy, animals will be divided into two replicate groups. The exposure start, FOB test day, and terminal necropsy day for the two groups will be staggered by a day. In order to provide consistent dosing conditions prior to neurobehavioral testing, both replicates will be exposed for the same number of days prior to FOB testing.

Generation of Test Substance: The test substance will be administered as an aerosol and vapor combination in the breathing air of the animals. The test atmosphere will be generated by a spray nozzle using procedures developed during pre-study trials and similar to those used for previous two-week and 2-day jet fuel studies conducted in Building 837. Trials will be performed to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels. The method will be described in the raw data of the study and in the report.

Exposure System: Rats will be exposed by inhalation in a Toxic Hazard Research Unit (THRU) chamber, which are 690 L whole-body exposure chambers. The chambers have a capacity of up to 32 rats held in THRU stainless steel wire mesh cage units. The THRU exposure chambers will be operated at flow rate of approximately 172 L/min to provide at least one complete air change in 4.0 minutes (15 air changes/hour; minimum guideline requirements

are 10 air changes per hour) and a T_{99} equilibrium time of approximately 18 minutes (T_{99} is the time for the concentration of test substance in the chamber to rise from background or zero to 99% of the equilibrium or target concentration). This chamber size and airflow rate is considered adequate to maintain an oxygen level that is at least 19%, the minimum required by the guidelines. At the end of an exposure, the chamber will be operated at approximately the same or higher flow rate using clean air. All rats will remain in the chamber for typically 30 minutes (but a minimum of the T_{99} equilibrium time), so that the bulk of the test material is cleared from the chamber. To minimize exposure to off-gassed test material, rats in the control chamber will be transferred from the chamber to their domiciliary caging and returned to the animal housing room in the vivarium prior to removing the test material exposed rats from their chambers. The test material-exposed rats will be held in a different animal room from the control rats to minimize exposure of the control animals to any off gassing from the exposed rats. All rats will be moved to an animal housing room in Building 838 during non-exposure periods.

Monitoring of Test Substance Concentration: A nominal exposure concentration will be calculated. The flow of air through the chamber will be monitored using appropriate, calibrated equipment. The test substance consumed during the exposure and the total volume of air passing through the chamber (volumetric flow rate times total exposure time) will be used to calculate the nominal concentration.

During the exposure, measurements of airborne concentrations will be performed in the animals' breathing zone. Aerosol concentration will be measured using a gravimetric filter or equivalent method. Vapor concentration will be measured using an appropriate sampling procedure and analytical method (Fourier Transform Infrared Spectrophotometry (FTIR, Nicolet 380, ThermoScientific, or equivalent). The analytical method will be developed in the pre-study trials and documented in the study file.

Particle Size Distribution: Particle size distribution measurement will be performed using an appropriate particle size instrument such as a cascade impactor (7-stage, In-Tox Products, Moriarity, NM or equivalent).

Uniformity: The distribution of material within the chamber will be checked for uniformity prior to the start of exposures. Measurements of concentration will be taken at 9 port locations under steady generation conditions.

Monitoring of Environmental Conditions: Chamber temperature, humidity, airflow rate, and static pressure will be monitored continuously and recorded at least three times during the exposure. Chamber temperature and relative humidity will be maintained, to the maximum extent possible, between 20 to 24°C and 30 to 70%, respectively.

The minimum frequency of chamber activity is summarized below:

TABLE 5: Summary of Chamber Activity

Activity	Minimum Frequency per chamber
Measured Test Substance Concentration	3 times per day
Aerosol concentration and size distribution	1 time per week
Temperature	3 times per day
Relative Humidity	3 times per day
Airflow Rate	3 times per day
Static Pressure	3 times per day
Nominal Test Substance Concentration (excluding the air control chamber)	1 time per day

In-Life Evaluation Observations

Viability Checks: Animals will be observed for morbidity, mortality, general appearance, and signs of severe toxic or pharmacological effects before and after exposures during exposure days. Animals will be observed at least once during the day during non-exposure periods. Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia.

Clinical Observations: Each animal will be examined at least twice pre-exposure, on the first day of exposure or one day prior, and weekly during the exposure regimen. Clinical observations will be recorded on scheduled body weight collection days. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, circulatory effects, autonomic effects, central nervous system effects, and reactivity to handling or sensory stimuli.

Ophthalmologic Examination: Ophthalmological examinations will be made on all animals prior to the administration of test substance and on high jet fuel concentration and control groups at termination. If changes in the eyes are detected, all animals in the other jet fuel concentration groups will be examined.

Body Weight: Body weights will be recorded the day after arrival and at randomization, weekly during exposures (one day prior, or the first day of exposures) and at necropsy.

Vaginal Cytology: During week 12 of the study, each day prior to the start of exposures, a vaginal lavage will be performed for each female rat. The lavage fluid will be placed on a glass slide and examined under a microscope to identify types of vaginal cells present. The predominance of any one specific type of cell is representative of a stage of the estrous cycle.

Motor Activity: After at least 11 weeks of exposure, on a non-exposure day following 5 consecutive days of exposure, all animals will be transferred into a neurotoxicology laboratory for motor activity assessment. Animals will be placed into standard clear polycarbonate cages and spontaneous motor activity will be determined using an automated photobeam data

collection system. Photobeams will record both fine movements (one photobeam broken) and ambulations (two photobeams broken in sequence). Data will be automatically collected by a computer system. Efforts will be made to control conditions that can affect behavior including sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions. Tests will be performed under reduced lighting conditions and with an approximately 70 dB white noise background to minimize disruption resulting from extraneous noise. A test session for most adult rats is anticipated to last for one hour in duration, and motor activity will be determined over individual 6 minute intervals.

Functional Observational Battery: Towards the end of the study, on a non-exposure day following 4 consecutive days of exposure, animals from one replicate group will be transferred into a neurotoxicology laboratory. The second replicate group will be transferred the next day. A functional observational battery (FOB) consists of non-invasive procedures designed to evaluate and document the absence or presence (or severity if appropriate) of a predetermined set of behavioral and clinical signs. Typically, observations are made: 1) while the rat is in an observation cage, 2) during removal of the rat from the observation cage, 3) while the rat is being held and examined for clinical observations, 4) as the animal moves freely about the open field, and 5) during manipulative tests. Typically, the observations proceed from the least to most manipulative tests to reduce the influence of handling on the rat's behavior. Efforts will be made to control conditions that can affect behavior including sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions.

The FOB includes, but is not limited to, the following observations, made and recorded in study records:

- In cage observations: Posture, tremors and spasms, and palpebral closure.
- Observations during removal from cage and handling: reactivity to handling, muscle tone, lacrimation, salivation, fur appearance, facial crust, breathing pattern, and other clinical signs.
- Open Field Observations: arousal and activity level, gait, body position, vocalization, tremor, spasm, unusual behaviors, urine and defecation count.
- Manipulative Observations:
    - approach response: response to a blunt object approaching and stopping before the animal’s nose.
    - acoustic response: response to a hidden metallic click.
    - tail pinch response: response to a pinch of the tail.
    - visual placement: response of forelimb to grasp for a surface while being held by the observer.
    - surface righting: righting response to being turned and briefly held on its back.
    - hind leg splay: response to being dropped approximately 30 cm (12 inches). Hind legs are painted to mark the location of the hind legs upon landing. This test is not done if animal is judged too weak to support its weight when dropped or if righting response is not displayed.
    - Grip strength: force necessary to break the animal’s grip on a wire mesh.
    - Pupil reflex: pupil response to light.
    - Body weight (if not weighed earlier on the day of the FOB).

Postmortem Observations

Moribund and Humane Euthanasia: Animals showing signs of severe debility, particularly if death appears imminent, will be euthanized to prevent unnecessary suffering or loss of tissues through autolysis. Necropsy should be performed immediately. If a necropsy cannot be performed on a euthanized animal or an animal found dead, the animal should be refrigerated to minimize autolysis. Necropsy should be performed within two days, if possible.

Terminal Euthanasia and Necropsy: Euthanasia and necropsy of all surviving animals will be performed on the day after the last scheduled exposure. Final body weights will be collected prior to euthanizing the animals.

Clinical Pathology: Blood will be removed via the vena cava following pentobarbital (IP) deep anesthesia. The blood will be used for hematology and clinical chemistry evaluations.

Gross Necropsy: A complete macroscopic examination will be performed on all animals, including all scheduled and unscheduled deaths. The postmortem examinations at the terminal necropsy shall be conducted by a veterinary pathologist.

Histopathology: Microscopic histopathological examination will be performed on the respiratory tract tissues including nasal airways (4 levels), trachea, larynx, and lungs (2 levels). Other organs to be examined include liver (2 levels), kidney (R and L), spleen, adrenals (R and L), heart and the other tissues listed above under gross necropsy. Preparation of histological slides and microscopic examinations will be performed by an outside contractor (to be identified) under GLP conditions.

Sperm Morphology: At necropsy, the caudal epididymis will be excised and weighed. Incisions will be made to release sperm into an appropriate medium. A small aliquot of the suspension will be stained with eosin Y and placed onto a slide. The slide will be examined for morphology (normal or abnormal) of the sperm.

V.1.2. Experiment 2 - Two-week Inhalation Genotoxicity Study of HRJ Fuel in Rats (*Rattus norvegicus*) using bone marrow micronucleus assay

Test Guideline: This genotoxicity study is based on the U.S. Environmental Protection Agency (U.S. EPA) Harmonized Test Guideline developed by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 870 870.5395 “Mammalian Erythrocyte Micronucleus Test.

Table 6: Two-week Inhalation of HRJ Fuel in Rats (Rattus norvegicus) with Genotoxicity Assay

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control	0	5	5
Low	200	5	5
Intermediate	700	5	5
High	2000	5	5
Negative Control	Saline	5	5
Positive Control	CP	5	5
Training	NA	5	5
Total		35	35

Concurrently with Experiment 1 (V.1.1.), animals (5 males and 5 females per exposure concentration, total of 40 animals) will be exposed to the same concentrations of HRJ fuel for approximately 2 weeks (10 exposures) for a bone marrow micronucleus assay to assess potential genotoxicity. There will be an additional 10 males and 10 females, not exposed to jet fuel, for use as positive and vehicle controls, for a total of 60 animals for the micronucleus assay. An additional group of 5 males and 5 female rats will be ordered for this study for training and method development for the micronucleus assay. Animals in the micronucleus study will be euthanized by IP injection of sodium pentobarbital, and dissected to remove the femur. The bone marrow will be processed and stained and then examined either microscopically or by flow cytometry.

Negative and Positive Controls: Concurrent negative control (physiological saline) and positive control, cyclophosphamide (CP), a known micronucleus inducer, will be included in the study. Animals in the negative control group will be used for the measurement of background frequency of micronucleated cells. Physiological saline at 1 ml per 100 g bodyweight will be given by IP injection, 24 hours before animal euthanasia and necropsy.

The positive control will be used to verify the responsiveness of the test system. A solution of CP in physiological saline will be prepared at 4 mg/ml. Based on the animal weight, the CP solution will be administered by IP injection at 1 ml/100 g body weight, to give a dose of CP of 40 mg / kg animal body weight, 24 hours before animal euthanasia and necropsy. Animals used for negative and positive controls in the micronucleus assay will be euthanized as described above.

Micronuclei Evaluation

Sample Processing: After euthanasia, animals on the micronucleus study will not undergo a full necropsy, but will have the femur removed. Bone marrow will be isolated by flushing the femur with 1 ml of heat-inactivated fetal bovine serum (FBS) into ice-cold 100% methanol fixative. Bone marrow cells will then be stained and analyzed according to the manufacturer's instructions (MicroFlow Plus RBM, Litron Laboratories, Rochester NY) by flow cytometry.

Micronuclei Observation: The frequency of micronucleated cells will be observed by flow cytometry from 20,000 reticulocytes per animal. The percentage of reticulocytes (%RET), micronucleated mature normochromatic erythrocytes (%MN-NCE), and micronucleated reticulocytes (%MN-RET) will be determined per animal. The results of the micronucleus assay can be considered positive if there is: a clear dose-related increase in the number of micronucleated reticulocytes; or a reproducible and statistically significant increase in the micronucleated reticulocyte frequency is detected for at least one concentration of the test substance; and a statistically significant difference in the micronucleated reticulocyte frequency between the positive and negative control.

V.2. Data Analysis

V.2.1 In Life and Postmortem Observation Statistics

The following items will be analyzed statistically in the final report (for male versus females) and control rats versus treated: feed consumption and weekly mean body weight values and body weight changes (from pretest) will be analyzed from In-Life Observations and hematology, coagulation, clinical chemistry, and organ weights from Postmortem Observations. Evaluation of equality of group means will be made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's (Bartlett, 1937) or Levene's test (Levene, 1960) will be performed to determine if groups have equal variances. If variances are equal, a standard one-way analysis of variance (ANOVA) will be used to assess significance. If variances are unequal, Welch's ANOVA will be used (Sokal and Rohlf, 1995). If significant differences among the means are indicated, additional tests will be used to determine which means are significantly different from the control: Dunnett's (Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). If data are non-normal, nonparametric methods such as Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953) will be used and if differences are indicated, Shirley's test (Shirley, 1977) or Steel's test (Steel, 1959) will be used to determine which means differed from control. Bartlett's or Levene's test for equality of variance will be conducted at the 1% significance level; all other statistical tests will be conducted at the 5% significance levels.

V.2.2. Motor Activity and FOB

For neurobehavioral assessments, the data for quantitative, continuous variables will be compared for the exposure and control groups by tests for homogeneity of variance, 2-way fixed effects (dose and sex) analysis of variance (ANOVA), and Dunnett's multiple comparison procedure for significant ANOVAs. If the ANOVA indicates statistical significance among experimental groups, the Dunnett's test will be used to delineate which groups differ from the

control group. A natural log transformation of the data will be used if the Levene's test indicates that the data are non-homogeneous. In the event that the Levene's test on the transformed data indicates non-homogeneous data, Welch's test will be used. A nested analysis of motor activity data will be performed using a repeated measures analysis with exposure as a grouping factor and interval as a within-subject factor. Additional exposure group comparisons of total cumulative test session activity will be performed. Incidence data will be compared using the appropriate statistical test, generally Fisher's Exact test. Incidence data for selected FOB endpoints with ordered severity scores will be analyzed for group differences using appropriate measures of association. Statistical analyses will be performed using either SAS or JMP statistical software or other statistical programs, as deemed appropriate. The probability value of less than 0.05 will be used as the critical level of significance for each statistical test, except that the critical level of significance for Levene's test for homogeneity of variance will be less than 0.01. All other uncorrected probability values of less than 0.05 will be listed in the report.

V.3. Laboratory Animals Required and Justification

V.3.1. Non-animal Alternatives Considered

There are still no adequate non-animal alternatives to *in vivo* inhalation studies. Toxicity assessments in cell lines to eliminate or reduce the use of animals exposed by inhalation have been conducted by various researchers. However, there is still little correlation between *in vitro* and *in vivo* studies of lung toxicity (Sayes, Reed and Warheit, 2007). Living animal models must still be used due to the complex nature of the lungs and potential systemic effects after exposure via inhalation.

V.3.2. Animal Model and Species Justification

The rat is used as a surrogate for humans in the detection of toxicity to establish occupational exposure levels. This rodent species is recommended for inhalation toxicity as directed by the U.S. EPA, Health Effects Test Guidelines, OPPTS 870.3465: 90-day Inhalation Toxicity and is preferred by the OECD guideline 413, Subchronic Inhalation Toxicity: 90-day Study. This study also follows the U.S. EPA Health Effects Test Guideline OPPTS 870.6200 Neurotoxicity Screening Battery which recommends the laboratory rat as the species of choice. Experiment 2 (see V.1.2.) follows the U.S. EPA Health Effects Test Guidelines OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test. This guideline recommends mice or rats, or any appropriate mammalian species. The experimental design for this protocol uses a rat model in the procedures and standards required by the current federal and international regulations. Historical control data are also available with this strain of rat for comparative evaluation.

V.3.3. Laboratory Animals

V.3.3.1. Genus / Species

Rattus norvegicus

V.3.3.2. Strain / Stock

Fischer F344

V.3.3.3. Source / Vendor

Charles River Laboratories, Wilmington MA

V.3.3.4. Age

At receipt: 6 weeks
At start of exposures: 8-9 weeks

V.3.3.5. Weight

Age appropriate

V.3.3.6. Sex

Males and females will be studied to determine if any observed effects are sensitive or specific to gender.

V.3.3.7. Special Considerations

Females will be nulliparous and nonpregnant

V.3.4. Number of Animals Required (by Species)
Rats: 150

V.3.5. Refinement, Reduction, Replacement (3 R's):

V.3.5.1. Refinement

The procedures used in this study were developed and refined by EPA scientists and non-EPA scientists with particular interests and/or expertise in inhalation toxicology studies and issued in OPPTS 870.3465: 90-day Inhalation Toxicity. The EPA has a process to revise guidelines if changes are needed and the currently published guidelines are being used for this study. These guidelines were also harmonized with Test Guideline 413, Subchronic Inhalation Toxicity: 90-Day Study, established by the Organization for Economic Cooperation and Development (OECD), an international organization composed of industrial nations with similar testing requirements for industrial chemicals. This study will use the OPPTS 870.3665 guidelines. The EPA guidelines do not specify a cage acclimation process. Rats will be acclimated to the stainless steel wire-mesh cages by placing them in the inhalation wire mesh cages for an increasing length of time (see V.1.1. Experiment 1 Quarantine and Acclimation Period).

V.3.5.2. Reduction

The number of animals selected is the minimum required to satisfy regulatory guidelines. We have added additional parameters for reproductive, neurobehavioral, and genotoxicity endpoints

to expand the screening ability of this study. If these additional endpoints are negative we will have eliminated the need for two additional animal studies.

V.3.5.3. Replacement

Currently there is no *in vitro* system that can substitute for an animal inhalation model. No non-animal systems are capable of addressing the research questions being asked in this protocol. There is still little correlation between *in vitro* and *in vivo* studies of lung toxicity (Sayes, Reed and Warheit, 2007). Non-mammalian systems, such as fish or amphibians are also unsuitable models as respiratory toxicology is strongly linked to respiratory physiology. The anatomical structure of the lung with the physiology of breathing used to bring inhaled air into the lungs causes specific patterns of deposition and interaction with respiratory tissue. This cannot be replicated in a water environment with fish, and the lung anatomy and interaction with inhaled air is sufficiently different in amphibians as to be a poor model for human inhalation studies. Animal systems, specifically the rat, are necessary for determining the repeated inhalation effect of HRJ fuel on the respiratory system.

V.4. Technical Methods

V.4.1. Pain / Distress Assessment

V.4.1.1. APHIS Form 7023 Information

V.4.1.1.1. Number of Animals

V.4.1.1.1.1. Column C: 0

V.4.1.1.1.2. Column D: 150

V.4.1.1.1.3. Column E: 0

V.4.1.2. Pain Relief / Prevention

V.4.1.2.1. Anesthesia / Analgesia / Tranquilization

Rats will be will be deeply anesthetized with sodium pentobarbital (intraperitoneal injection, at 30–40 mg/kg, using a 2.5 or 3 ml syringe with a 20 or 22 gauge needle) before the blood collection procedure. Pain alleviation of anesthetized animals will be determined by testing the toe pinch reflex. When a rat is no longer responsive to toe pinch it will be considered adequately anesthetized.

V.4.1.2.2. Pre- and Post-procedural Provisions

The PI will notify the Attending Veterinarian, at least one week in advance, of all procedures that require the use of anesthesia to ensure the Attending Veterinarian's availability in the event he/she is needed.

Viability Checks: Animals will be observed for morbidity, mortality, general appearance, and signs of severe toxic or pharmacological effects before and after exposures during exposure days by the inhalation staff. Animals will be observed twice daily for signs of distress and the observations will be recorded by RSC staff. Animals will be observed at least once during the day during non-exposure periods by the RSC staff. See section V.5.2.1.

Clinical Observations: Each animal will be examined at least twice pre-exposure, on the day of each exposure, and once on the weekend between exposures during the thirteen week study period. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, circulatory effects, autonomic effects, central nervous system effects, and reactivity to handling or sensory stimuli.

Display of a physical condition or state that suggests consideration of euthanasia before the study endpoints have been developed by the Organization of Economic Cooperation and Development (OECD) (2000) and are also referenced in WPAFB IACUC Policy 01-06. The OECD clinical signs to be used as early study endpoints are listed below:

- abnormal vocalization
- abnormal aggressiveness
- abnormal reaction to handling
- abnormal external appearance (i.e., ruffled fur indicating lack of grooming, dried urine and/or feces near anogenital area)
- prolonged, impaired ambulation preventing the animal from reaching food or water, or prolonged anorexia
- excessive weight loss and/or extreme emaciation and/or severe dehydration
- evidence to suggest irreversible organ failure
- prolonged absence of voluntary responses to external stimuli
- persistent, difficult labored breathing
- prolonged inability to remain upright
- persistent convulsions
- self-mutilation, open wounds, or skin ulceration
- prolonged diarrhea

Animals consistently displaying one or more of these signs will be immediately removed from the study (i.e., euthanized). Persons (RSC and NAMRU–Dayton staff) observing any animals displaying the indicated clinical signs will immediately inform the study PI. The PI will authorize NAMRU–Dayton or RSC staff to euthanize the animal. During off-duty hours, if PI is not able to be contacted via phone or email, the PI authorizes the RSC staff, at the discretion of the Attending Veterinarian, to euthanize any animal that is found moribund, or appears to be in intense, unrelievable pain. The carcass of the animal will be placed in a plastic bag along with its cage card. The bag will then be placed in the walk-in refrigerator in Necropsy, room 67, Bldg 838. The RSC staff will then alert the PI by email as to the final condition of the animal and the animal number on the cage card.

V.4.1.2.3. Paralytics

N/A

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures

V.4.1.3.1. Databases Searched

Dialog was used to search the following databases: AGRICOLA, Dissertation Abstracts Online, BIOSIS, Inside Conferences, EMBASE, International Pharmaceutical Abstracts, IPA Toxicology, MEDLINE, ToxFile, BIOSIS Toxline, FEDRIP. The Defense Technical Information Center (DTIC) Biomedical Research Database, Research Summaries and Technical Reports, IR & D were searched. Also searched other websites and databases: Animal Welfare Information Center (AWIC) web site and Johns Hopkins Center for Alternatives to Animal Testing (Altweb/Center for Alternatives to Animal Testing), Bibliography on Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing (Altbib), Non-animal Methods for Toxicity Testing (AltTox), Cambridge Scientific Abstracts and Engineering Village.

V.4.1.3.2. Number, Date, and Resources of Search

Literature search 2011152 was completed 12/27/2010 by Carol Reed, MLIS of the D'Azzo Research Library, Det 1, AFRL/WSC.

V.4.1.3.3. Period of Search

All years for each database

V.4.1.3.4. Key Words of Search

pain, alternatives, distress, rat, mouse/mice, micronucleus, inhalation, acute, two-week, 90-day, subchronic, 13-week, sensory irritation (in lungs so respiratory tract), RD50, Alarie test, *in vitro*, cell culture plus kerosene, Jet A, jet fuel, JP-8, HRJ, hydrorenewable jet, HRJ Camelina, HRJ plant oils, HRJ Tallow, HRJ Animal Fats and Oils, biofuel, biobased/bio-based

V.4.1.3.5. Results of Search

The current literature search did not yield any alternatives to the 13-week inhalation study. From a search for a previous protocol, one study was found that tried to correlate *in vivo* mouse inhalation exposures to JP-8 with *in vitro* rat lung slices exposed to JP-8 in cell culture medium (Hays, Lantz, and Witten, 2003). The *in vitro* study was not conducted concurrently with the *in vivo* study, so similar cellular pathology seen between the two studies was a qualitative correlation. The study was not proposed as a substitute for an *in vivo* inhalation study. Additionally, it is not possible to extrapolate no-effect doses to humans in order to establish occupational exposure levels. So there is still little correlation between *in vitro* and *in vivo* studies of lung toxicity (Sayes, Reed and Warheit, 2007). The procedures used in a 90-day inhalation study do not normally produce pain or distress; there is only the potential for pain if a test agent produces significant irritation.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification

N/A

V.4.2. Prolonged Restraint

N/A

V.4.3. Surgery

V.4.3.1. Pre-surgical Provisions

Rats will be deeply anesthetized with sodium pentobarbital (intraperitoneal injection, at 30–40 mg/kg, using a 2.5 or 3 ml syringe with a 20 or 22 gauge needle) and exsanguinated by transection of the abdominal aorta after collecting blood for clinical pathology. Terminal surgery will be performed using surgical gloves, mask, and clean instruments. Needles and syringes will not be treated with an anticoagulant.

V.4.3.2. Procedure(s)

Following anesthesia per section V.2.1.2.1, the abdomen will be opened with scissors, the diaphragm will be cut (to create a pneumothorax), the intestines moved to one side, and blood will be drawn from the caudal vena cava or heart using a 5 mL syringe with a 21-23 gauge, ½-1" needle. Needles and syringes will not be treated with an anticoagulant.

V.4.3.3. Post-surgical Provisions

The surgical procedure is terminal.

V.4.3.4. Location

Anesthesia, euthanasia, and tissue harvest will be performed in the RSC, room 67. Behavioral observations will take place in room 259 or 269 of Building 837.

V.4.3.5. Surgeon

All protocol personnel listed may perform the terminal surgery procedures. All inexperienced personnel will be supervised and trained by senior, experienced protocol personnel, and/or RSC staff.

V.4.3.6. Multiple Major Survival Operative Procedures

V.4.3.6.1. Procedures

None

V.4.3.6.2 Scientific Justification

N/A

V.4.4. Animal Manipulations

V.4.4.1. Injections

In Experiment 2, animals in the negative and positive control groups will be administered either physiological saline (1 ml per 100 g body weight) or cyclophosphamide (CP) in physiological saline (4 mg/ml, 40 mg / kg animal body weight) by intraperitoneal injection. Each animal will receive a single injection using a 20 or 22 gauge needle. Injections are part of the euthanasia

protocol (See Section V.4.6). No other injections are planned. Blood will be drawn from the caudal vena cava or heart using a 5 mL syringe with a 21-23 gauge ½-1" needle. Needles and syringes will not be treated with an anticoagulant.

V.4.4.2. Biosamples

Following anesthesia and surgery as described in sections V.4.1.2.1 and V.4.3., 3-5 mL of blood will be collected from the caudal vena cava or heart using a 5 ml syringe with a 21-23 gauge, ½-1" needle. Needles and syringes will not be treated with an anticoagulant. Following blood draw the rat will be rapidly decapitated with a rat guillotine.

V.4.4.3. Adjuvants

N/A

V.4.4.4. Monoclonal Antibody (MAb) Production

N/A

V.4.4.5. Animal Identification

Each animal will be assigned a temporary identification number and cage location upon receipt. After selection for study, each animal will be identified by tail tattoo with a number assigned by the Testing Facility. The study number plus the number assigned by the Testing Facility will comprise the unique animal number for each animal. A cage assignment chart will indicate cage assignment by the animal identification number. Animals used in FOB testing will be assigned a temporary identification number by cage cards for the purpose of keeping the observers blind to treatment given.

V.4.4.6. Behavioral Studies

Formal observation of animal behavior as described above for motor activity and functional observational battery are specified endpoints for this study. The U.S. EPA Health Effects Test Guideline OPPTS 870.6200 "Neurotoxicity Screening Battery" describes the specific observations to be made. These endpoints are intended to identify unusual or abnormal behavior that may indicate potential neurotoxic effects caused by the inhalation of HRJ fuel.

V.4.4.7. Other Procedures

None.

V.4.4.8. Tissue Sharing

Tissues not needed for data analysis will be made available to WPAFB researchers upon request.

V.4.5. Study Endpoint

The endpoint for all animals in this study will be euthanasia as described in section V.4.6.

V.4.6. Euthanasia

Following anesthesia as described in section V.4.1.2.1, the abdomen will be opened with scissors, the diaphragm will be cut (to create a pneumothorax), the intestines moved to one side, and blood will be drawn from the caudal vena cava or heart using a 5 mL syringe with a 21-23 gauge ½-1" needle. Rapid decapitation will immediately follow blood draw. Euthanasia can/will occur at any of these steps.

V.5. Veterinary Care

V.5.1. Husbandry Considerations

Upon arrival at Bldg 838, WPAFB Area B, animals will be housed, fed, and watered in accordance with RSC SOP 603. New animals will be segregated from the current population for a quarantine and acclimation period of 7 to 10 days. Animal rooms will be maintained at a temperature and relative humidity in accordance with the recommendations of the NRC's Guide for the Care and Use of Laboratory Animals, with approximately 15 complete air changes per hour, and a 12hr:12hr electronically controlled light:dark cycle. Animal caging will be cleaned in accordance with the above SOPs, and all animals will be observed twice daily by RSC personnel for any signs of pain, distress, or any other abnormalities.

V.5.1.1. Study Room

All inhalation exposures will be conducted in room 264 of building 837. Behavioral observations will take place in room 259 or 269 of Building 837.

V.5.1.2. Special Husbandry Provisions

Wire-bottom cages are used in whole-body inhalation chambers to ensure that the exposure atmosphere in the chamber is uniformly distributed to all cage units so that all rats will be exposed to the same concentration of test material.

V.5.1.3. Exceptions

V.5.2. Veterinary Medical Care

V.5.2.1. Routine Veterinary Medical Care

Animals will be observed twice daily for signs of distress and the observations will be recorded by RSC staff. The PI will be contacted if an animal is discovered in a moribund condition or appears to be in intense pain during duty hours (0730-1600, Monday-Friday). At that time, the PI will consult with the Attending Veterinarian as to appropriate actions to be taken for the well-being of the animal. If any unexpected animal deaths occur, the PI will immediately notify the Attending Veterinarian (or alternate) for consultation as to the cause of death, any immediate corrective actions to institute, and the need for a necropsy.

V.5.2.2. Emergency Veterinary Medical Care

During normal duty hours, animal health care emergencies should be reported to the RSC Facility Manager (937-255-7210) or Attending Veterinarian (937-255-8510). After normal duty hours, weekends, and holidays, animal health care emergencies should be reported as described in the memorandum document “Emergency Veterinary Medical Care” describing procedures for contacting emergency personnel. This document is posted on the bulletin board across from the Attending Veterinarian’s office (Room 59). During off-duty hours, the PI will authorize the RSC staff, at the discretion of the Attending Veterinarian, to euthanize any animal that is found moribund, or appears to be in intense, unrelievable pain. The carcass of the animal will be placed in a plastic bag along with its cage card. The bag will then be placed in the walk-in refrigerator in Necropsy, room 67, Bldg 838. The RSC staff will then alert the PI by email as to the condition of the animal and the animal number on the cage card.

V.5.3. Environmental Enrichment

V.5.3.1. Enrichment Strategy

Animals will be individually housed in the exposure chambers to enable the free flow of test atmosphere around the animal. Animals that are group housed may huddle together, acting as a filter to remove test material from the atmosphere. For ease of transport and transferring animals between domiciliary housing and exposure cage units, animals will also be singly housed in their domiciliary housing. Enrichment items (e.g., nylabones) may be used in domiciliary cages.

Enrichment will be provided by the WPAFB RSC staff in accordance with RSC SOP 603.

V.5.3.2. Enrichment Restrictions

Enrichment items (e.g., nylabones) may not be permitted in exposure chambers as they could interfere with the free flow of the exposure atmosphere. Additionally, HRJ fuel droplets could deposit on the enrichment items and be ingested as the animal chews on it. It is desirable to limit the ingestion of the test substance as much as possible.

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING

All personnel involved in the protocol have attended the WPAFB Investigator Training Course, or are scheduled to take the next available one offered.

Activity	Name	Qualifications	Training
Animal handling, blood draw, euthanasia, tissue harvest	William Howard, PhD	PhD, Biochemistry, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB animal handlers training.
Animal handling, blood draw, euthanasia, tissue harvest	Brian Wong, PhD	PhD, Environmental Engineering Science, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, clinical evaluations, blood draw, euthanasia, tissue harvest	Michael Gargas, PhD	PhD, Biomedical Sciences, Toxicology specialty, >25 years experience in animal handling and experimentation	WPAFB animal handlers training.

Activity	Name	Qualifications	Training
Animal handling, clinical evaluations, euthanasia, tissue harvest	Chet Gut, MS	MS, Pharmacology and Toxicology, >2 years live animal handling experience, including clinical observations, neurobehavioral testing, anesthesia, euthanasia, necropsy, and tissue harvest	WPAFB animal handlers training
Animal handling, clinical evaluations, blood draw, euthanasia, tissue harvest	Michelle Okolica, BS	BS, Biological Sciences, > 13 years live animal handling of mammals, birds, and reptiles. Five years combined professional euthanasia necropsy, and tissue harvest experience with birds and mammals	WPAFB animal handlers training
Animal handling, clinical evaluations, blood draw, euthanasia, tissue harvest	Sue Prues, BA	BA, Biology, combined total of 23 years doing biomedical research many projects involve the handling and husbandry required for research animals	Purina Laboratory Animal Care Course Certification. WPAFB animal handlers training
Animal handling, clinical evaluations, blood draw, euthanasia, tissue harvest	Shawn McInturf, MA	MA. Applied Behavioral Science, >12 years practical animal handling experience. Trained in anesthesia (CO_2), euthanasia (to include guillotine), pup handling and manipulation, and rodent necropsy. Expert in conducting animal neurobehavior testing (NHRC NTAB WPAFB protocol).	WPAFB animal handlers training. RCRA training (biohazards and chemical)
Animal Handling, clinical evaluations	Arden James, BA	BA, Biology, >29 years of experience in basic research and laboratory management of inhalation toxicology studies using rodents or non-human primates exposed to test chemicals by nose only, intratracheal or whole body inhalation procedures.	Various laboratory training courses including intra-laboratory Annual Animal Care and Handling and Animal Care and Use; also on-the-job training loading rodents in nose only exposure tubes or whole body wire mesh cages for inhalation exposures.

Activity	Name	Qualifications	Training
Animal handling, dinical evaluations	Jim Reboulet, MS	MS, Chemistry, >18 years experience in biomedical research using laboratory animals including mice, guinea pigs and rats	Purina Laboratory Animal Care Course – NMRI/TD – 1994. On the job training loading rats into nose only exposure tubes and whole body exposure cages and chambers – from Dr. E.C. Kimmel –1990 to present. Administration of CO_2 for anesthesia and euthanasia - from Dr. Kimmel - 1993 to present.
Animal handling, blood draw, euthanasia, tissue harvest	Pedro Ortiz, PhD	PhD, Molecular Genetics and Microbiology, > 5 years animal handling experience including clinical observations, neurobehavioral testing, anesthesia, euthanasia, necropsy, and tissue harvest	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Karen Mumy, PhD	PhD, Microbiology, > 5 years animal handling experience including clinical observations, neurobehavioral testing, anesthesia, euthanasia, necropsy, and tissue harvest	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Andre Ntamack, PhD	PhD, Biochemiistry and Metabolism, > 5 years animal handling experience including clinical observations, neurobehavioral testing, anesthesia, euthanasia, necropsy, and tissue harvest	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Tracy Doyle – McInturf, BA	BA, Microbiology, > 5 years animal handling experience including clinical observations, neurobehavioral testing, anesthesia, euthanasia, necropsy, and tissue harvest	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Richard Erickson, MS	MS, Environmental Health, Industrial Hygiene, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course

Activity	Name	Qualifications	Training
Animal handling, blood draw, euthanasia, tissue harvest	Dan Hardt, MS	MS, Environmental Engineering, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Lisa Sweeney, PhD	PhD, Chemical Engineering, minor Toxicology, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Michael Grimm, BS	BS Biomedical Engineering, limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Angie Hulgan	limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Brian Sharits	limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Jessica Sharits	limited animal handling experience and will only work under the supervision of the more experienced study personnel	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Palur Gunasekar, PhD	PhD, Endocrinology, >20 years animal handling experience including dosing, handling, surgery of small animals, isolation of major body organs, blood collection through cardiac and vein puncture, in-life animal manipulations, tissue harvest and euthanasia.	WPAFB Investigator Training Course
Animal handling, blood draw, euthanasia, tissue harvest	Andrew Osterburg, PhD	PhD, Biology, training as a cell biologist with >6 years experience in handling small animals including IP injections, gavage, necropsy, and euthanasia.	WPAFB Investigator Training Course

Activity	Name	Qualifications	Training
husbandry, animal care, animal handling, clinical evaluations, viability, body weights, euthanasia, tissue harvest	Dick Godfrey	Mr. Godfrey has 38 years of laboratory animal research experience and is certified as a Lab Animal Technologist through AALAS. Mr. Godfrey is highly experienced in Toxicology studies and is proficient in all forms of dosing, blood draws, injections and methods for anesthesia, euthanasia, and necropsies.	Certified AALAS Animal Technologist - 1980 Laboratory Animal Medicine and Science Series - 1980 Purina Animal Care WPAFB animal handlers training RCRA training (biohazards and chemical)
husbandry, animal care, animal handling, clinical evaluations, viability, body weights, euthanasia, tissue harvest	Tim Bausman, BS	Mr. Bausman has a BS in Education and 32 years of laboratory animal research experience in Reproductive Toxicology studies and is proficient in all forms of dosing, blood draws, injections and methods for anesthesia, euthanasia, and necropsies.	Certified AALAS Lab Animal Technologist Certified X-Ray Technologist WPAFB animal handlers training Purina Laboratory Animal Care Course Certification RCRA training (biohazards and chemical)

VII. BIOHAZARDS/SAFETY:

Personal protective equipment such as gloves, goggles or safety glasses, and lab coats will be worn when handling HRJ fuel and rats.

VIII. ENCLOSURES

VIII.1.1 Enclosure 1: References

VIII.2 Enclosure 2: HRJ Fuel Information
- **VIII.2.1:** HRJ HSAF
- **VIII.2.2:** HRJ MSDS

VIII.3: Cyclophosphamide Information
- **VIII.3.1:** Cyclophosphamide HSAF
- **VIII.3.2:** Cyclophosphamide MSDS

IX. ASSURANCES

PROTOCOL TITLE: 90-Day Inhalation Toxicity Study of HRJ Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard / Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical, and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R", namely, "Responsibility," which the DOD has embraced for implementing animal use alternatives where feasible and conducting humane and lawful research.

G. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review and is consistent with good scientific research practice.

H. Painful Procedure(s): (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress WILL/WILL NOT be relieved with the use of anesthetics, analgesics, and/or tranquilizers. I have considered alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

William Howard (Principal Investigator Printed Name) William R. Howard (Principal Investigator Signature) 21 MAR 2011 (Date)

I. **OCCUPATIONAL HEALTH PROGRAM:** I acknowledge the inherent risks associated with animal contact, such as allergies and zoonoses. I have made a reasonable, good faith effort to ensure all persons with animal contact, working on this protocol are enrolled in an Occupational Health Program.

Name	Enrollment Date	Provider
Michael Gargas	02/2010	Base Occ Health
Chet Gut	06/2010	Kettering Worker's Care
Michelle Okolica	12/2010	WorkCare/Concentra
Sue Prues	12/2010	WorkCare/Concentra
Shawn McInturf	01/2011	WorkCare/Concentra
Arden James	01/2010	WorkCare/Concentra
Jim Reboulet	01/2011	WorkCare/Concentra
Pedro Ortiz	06/2009	Base Occ Health
Karen Mumy	01/2011	WorkCare/Concentra
Andre Ntamack	09/2010	Base Occ Health
Tracy Doyle-McInturf	01/2011	WorkCare/Concentra
Richard Erickson	09/2009	Base Occ Health
Dan Hardt	09/2010	Base Occ Health
Lisa Sweeney	09/2010	WorkCare/Concentra
Brian Wong	09/2010	WorkCare/Concentra
Michael Grimm	07/2010	WorkCare/Concentra
Angie Hulgan	07/2010	Kettering Worker's Care
Brian Sharits	07/2010	Kettering Worker's Care
Jessica Sharits	07/2010	Kettering Worker's Care
Palur Gunasekar	09/2010	Base Occ Health
Dick Godfrey	04/2010	MedWorks
Tim Bausman	04/2010	MedWorks

William Howard (Principal Investigator Printed Name) William R. Howard (Principal Investigator Signature) 21 MAR 2011 (Date)

Enclosure 1

REFERENCES

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WPAFB HAZARDOUS AGENT SUMMARY FORM

If the proposed study will involve the use of hazardous or potentially hazardous agents, please provide the following information. This form must be filled out for each chemical used in a study. Material Data Safety Sheets (MSDS), (if available) must be attached to each Hazardous Agent Summary Form, (HASF).

Chemical Name:(common Name) HRJ Fuel

Estimated highest dose level 2000 mg/m3

(Exposure Concentration): 2000 mg/m3

Route(s) of administration: Inhalation

Duration of treatment: 6 h/day, 5 d/week, 65 exposure days

Estimated maximum length of time animals will be monitored following exposure:

Estimated exposure time for personnel: 1 hr

Estimated exposure concentration for personnel: 0 mg/m3

The following personal protective equipment (PPE) must be worn/used in the animal rooms. PPE must be removed before leaving the animal rooms.

- □ Disposable gowns
- √ Lab coats
- √ Disposable gloves (type) Nitrile
- √ Safety glasses (type)
- □ Goggles (type)
- □ Respirator (type)
- □ Scrubs
- □ Face Shields
- √ Shoe Covers
- □ Bouffant Cap
- □ Waterproof Boots
- □ Others

PI will inform personnel on proper processes, procedure and equipment needed to work safely with this chemical. Provide information below:

HRJ fuel will be generated as and aerosol/vapor mixture into closed, whole body exposure chambers. Personnel should not be exposed to the test atmosphere. Chambers will be flushed with clean air prior to opening for animal care or inhalation activities. Personnel may be briefly exposed to fumes off-gassing from the chamber walls or from animal fur. Personnel will wear labcoats, gloves, safety glasses,and shoe covers when working with the animals in the exposure room

Health risks associated with this chemical: Tissue Irritation
Exposure routes: Dermal, Inhalation, ingestion
Other controls: keep away from high temperatures or ignition sources, keep container closed

Principal Investigator:

Signature William R. Howard Print Name William R. Howard Date 2/10/11

Environmental, Safety Officer, Air force:

Signature Kathy Kincaid Print Name KATHY KINCAID Date 2/10/11

Environmental, Safety Officer, Navy:

Signature Keith E. Johnson Print Name KEITH E. JOHNSON Date 10 FEB 2011

MSDS 211084

uop
A Honeywell Company

MATERIAL SAFETY DATA SHEET

1. CHEMICAL PRODUCT AND COMPANY INFORMATION

Product Name: **Bio-oil Derived SPK (C100 in process)**

Product Use: Chemical - Fuel (Experimental)

NOTE: This sample is for research and development purposes only. The handling and use of this material must be supervised by qualified individuals. The chemical, physical and toxicological properties of this material have not been fully investigated. Use due precaution in handling, storage and disposal.

UOP LLC
25 E. Algonquin Road
Des Plaines, IL 60017-5017
USA
Tel: +1-847-391-3189
Fax: +1-847-391-2953

UOP Ltd.
"Licngate", Ladymead
Guildford, Surrey GU1 1AT
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Emergency Assistance - 24 hour Emergency Telephone Numbers:

USA (UOP LLC) :	+ 1-847-391-2123
USA (CHEMTREC) :	+ 1-800-424-9300
Canada (CANUTEC) :	+ 1-613-996-6666
Outside USA (CHEMTREC) :	+ 1-703-527-3887

2. HAZARDS IDENTIFICATION

Emergency Overview:
The product is considered harmful via ingestion. Avoid breathing the product. Keep away from heat, sparks, and flame. The product is combustible and toxic vapors may be given off in a fire.

Form: Liquid
Color: Colorless

Potential Health Effects:

Primary Routes of Exposure: Contact with skin, eyes and inhalation of product vapor. Product ingestion is unlikely to occur if proper safety/hygiene procedures are followed.

Eye Contact: Repeated or prolonged exposure may cause eye irritation.

Skin Contact: Causes mild skin irritation.

Ingestion: May be harmful if swallowed. Aspiration can be a hazard if this material is swallowed.

Inhalation: Inhalation of product vapors or mist may cause irritation of the respiratory system. May cause Central Nervous System effects.

Chronic Effects: None known.

Carcinogenicity Classification:

International Agency for Research on Cancer (IARC):
Neither the product nor the components are classified.

U.S. National Toxicology Program (NTP):
Neither the product nor the components are classified.

U.S. Occupational Safety and Health Administration (OSHA):
Neither the product nor the components are classified or regulated.

American Conference of Governmental Industrial Hygienists (ACGIH):
Neither the product nor the components are classified.

3. COMPOSITION/INFORMATION ON INGREDIENTS

INGREDIENT & CAS NO.	% WEIGHT	ACGIH TLV-TWA	OSHA PEL-TWA	UNITS
C9 - C15 paraffin bio-oil derived	100% >99	200	N.E.	mg/m^3

Abbreviations:

N.A.	- Not Applicable	RD	- Respirable Dust	Fu	- Fume	IS	- Insoluble
N.E.	- None Established	R	- Respirable Fraction	I	- Inhalable	FuD	- Fume and Dust
STEL	- Short Term Exposure Limit	F	- Respirable Fibers	TD	- Total Dust	SC	- Soluble Compounds

4. FIRST AID MEASURES

Eye contact: Flush immediately with plenty of water, also under the eyelids, for at least 15 minutes. Consult a physician.

Skin contact: REMOVE FROM SKIN IMMEDIATELY. Take off all contaminated clothing immediately. Remove adhering matter immediately. Use waterless hand cleaner. Then wash with lots of water and soap. Consult a physician.

After inhalation: Remove the victim into fresh air. If symptoms persist, call a physician.

After ingestion: Do not induce vomiting. Call a physician immediately.

Notes to physician: In the unlikely event that large quantities of the product are ingested, gastric lavage should be considered. Aspiration into the lungs may cause chemical pneumonia. An activated charcoal slurry taken within 30 minutes of product ingestion may reduce the toxicity of the chemical. A 5:1 ratio of charcoal to material ingested is the recommended dosage. Activated charcoal should not be considered as an antidote; normal symptomatic treatment is recommended with or without the administration of activated charcoal.

5. FIRE FIGHTING MEASURES

Suitable extinguishing media: Water spray. Foam. Dry chemical. Carbon dioxide (CO_2).

Unsuitable extinguishing media: Do not use a solid water stream as it may scatter and spread fire.

Fire and explosion hazards: In the event of fire and/or explosion do not breathe fumes. Cool containers / tanks with water spray. Heating/burning can release hazardous gases: carbon oxides (CO, CO_2) and various hydrocarbons.

Special protective equipment: Wear protective clothing. In case of respirable dust and/or fumes, use self-contained breathing apparatus.

Flash Point: >100°F (>38°C)

6. ACCIDENTAL RELEASE MEASURES

Personal protection: See Section 8.

Environmental precautions: Prevent product from entering drains. Do not flush into surface water or sanitary sewer system. Avoid subsoil penetration.

Clean-up: Remove all sources of ignition. Stop leak at source. Keep people away from and upwind of spill/leak. Contain material using temporary measures such as sand bags, booms or adsorbent socks. Soak up with inert absorbent material (e.g. sand, silica gel, universal binder, sawdust). Never use spilled product.
Spilled product should be disposed of in accordance with all applicable government regulations. (See Section 13).
Small amounts: Soak up with inert absorbent material and dispose of in accordance with applicable regulations.

7. HANDLING AND STORAGE

Handling: Use only in well-ventilated areas. Wear personal protective equipment. In case of insufficient ventilation, wear suitable respiratory equipment (see Section 8 of MSDS). Keep away from open flames, hot surfaces and sources of ignition.

Storage: Keep containers tightly closed in a cool, well-ventilated place. Store in original container. Keep away from heat and sources of ignition.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering measures: Ensure adequate ventilation, especially in confined areas.

Personal protection equipment: Avoid contact with skin, eyes and clothing. Handle in accordance with good industrial hygiene and safety practice.

Eye protection: Tightly fitting safety goggles. Face-shield.

Hand protection: Solvent-resistant gloves.

Skin and body protection: Solvent-resistant apron and boots. Protective suit. Remove and wash contaminated clothing and gloves, including the inside, before re-use.

Respiratory protection: In case of insufficient ventilation, wear suitable respiratory equipment. Air-purifying respirator with NIOSH classification N-100 filter or P-100 (or equivalent) if oil/liquid aerosols are present (42 CFR 84).

9. PHYSICAL AND CHEMICAL PROPERTIES

These data do not represent technical or sales specifications.

Form: Liquid

Color: Colorless

Odor: Odorless to mild paraffin

pH: N.A.

Boiling point/range: 298-572°F (148-300°C)

Melting point/range: N.A.

Flash point: >100°F (>38°C)

Autoignition temperature: N.D.

Bulk density: N.D.

Explosion limits: N.A.

Vapor pressure: N.D.

Relative density/Specific Gravity: 0.75 - 0.80 g/ml @ 15°C

Vapor density: >1

Viscosity: N.D.

Water solubility: Insoluble

Solubility: N.D.

Abbreviations:

N.D. - Not Determined
N.A. - Not Applicable

10. STABILITY

Stability: Stable at normal conditions. Decomposes on heating.

Hazardous decomposition products: No decomposition if used as directed. Under conditions giving incomplete combustion, hazardous gases produced may consist of carbon oxides (CO, CO_2) and various hydrocarbons.

Conditions/Materials to avoid: Keep away from ignition sources. Oxidizing agents.

11. TOXICOLOGICAL INFORMATION

Acute toxicity:

LD50/oral/rat: No data available.

LD50/dermal/rabbit: No data available.

LC50/inhalation/rat: No data available.

Chronic toxicity: Classification of Ingredients

EC Carcinogenic:
Not listed.

Carcinogenicity (ACGIH):
Not listed.

EC Mutagenic:
Not listed.

IARC classification:
Not listed.

EC Toxic for Reproduction:
Not listed.

Routes of exposure: Exposure may occur via inhalation, contact with skin and eyes.

Irritation:

Skin (rabbit): No data available.

Eye (rabbit): No data available.

Additional product information:
Avoid repeated and prolonged exposure.

Additional component information:
No data available.

12. ECOLOGICAL INFORMATION

Mobility:
No data available.

Biodegradation:
No data available.

Bioaccumulation:
No data available.

Aquatic toxicity:
No data available.

Further Information:
No information available.

13. DISPOSAL CONSIDERATIONS

Provisions relating to waste: EPA - Resource Conservation and Recovery Act (RCRA) Hazardous and Solid Waste Management Regulations.

Disposal information: Dispose of in compliance with all applicable regulations. Waste material may exhibit the U.S. EPA's RCRA hazardous waste characteristic of Ignitability (D001) if representative sample of the waste has a flash point of less than 140°F (60°C).

14. TRANSPORT INFORMATION

UN-No.:	Proper shipping name:	Packing group:
UN1863	Fuel, aviation, turbine engine	III

Transport Mode	*Class*	*Additional Information*		*Remarks*
U.S. DOT:	3	Reportable Quantity:	N.A.	N.A.
		Marine Pollutant DOT:	No	
ADR/RID:	3	Danger Code:	30	N.A.
IMDG:	3	Marine pollutant:	No	N.A.
		EmS:	F-E, S-E	
IATA:	3	Instr. Passenger:	309/Y309	N.A.
		Instr. Cargo:	310	

15. REGULATORY INFORMATION

United States

Toxic Substances Control Act (TSCA): The TSCA Inventory status has not been determined for the ingredient(s) of this product.
This material must be used in compliance with the TSCA Research and Development Exemption requirements (40 CFR 720.36). Regulations require: (1) All persons engaged in experimentation, research, or analysis on this substance, including manufacture, processing, use, transport, storage, and disposal associated with R&D activities, are notified of all health risk information; (2) The adequacy of the notification to all such persons is ensured by a technically qualified individual; (3) Activities of all such persons are supervised by a technically qualified individual; (4) All areas in which exposure may occur are conspicuously labeled; (5) Any information, test data, or indication of significant adverse reactions by persons exposed to the substance be evaluated to determine whether there is risk to health or the environment which may reasonably be associated with such exposure; (6) Prudent laboratory practices are followed, if the activity is in a laboratory.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) Reportable Quantity:
The following component(s) of this product is/are subject to release reporting under 40 CFR 302 when release exceeds the Reportable Quantity (RQ):
-- None --

CWA (Clean Water Act):
Any spill or release of this product to navigable waters or adjoining shoreline sufficient to cause a sheen or deposit of a sludge or emulsion is subject to the Discharge of Oil Notification requirements under 40 CFR 110.6.

SARA Title III (Superfund Amendments and Reauthorization Act of 1986):
Section 302 (Extremely Hazardous Substances):
The following component(s) of this product is/are subject to the emergency planning provisions of 40 CFR 355 when there are amounts equal to or greater than the Threshold Planning Quantity (TPQ):
-- None --

Section 313 (Toxic Chemicals):
The following component(s) have been specified as Toxic Chemicals under SARA Section 313 and may be subject to the Toxic Release Inventory (TRI) reporting requirements under 40 CFR 372:
-- None --

The following components are listed in U.S. State Regulations:

State Reg Reference:	State Reg Reference:
California - Proposition 65:	None.
Massachusetts Right-To-Know:	Kerosene
New Jersey Right-To-Know:	Kerosene
Pennsylvania Right-To-Know:	Kerosene

Note: Other U.S. State Regulations may exist, check your local sources if available or contact the UOP Product Stewardship Manager (see Section 16).

Canada

Canadian Hazardous Products Act:
Not determined.

Canadian Environmental Protection Act: Not determined.

European Union (EU)

European Inventory of Existing Commercial Chemical Substances: Not determined.

Council of European Communities Directive on Classification, Packaging and Labelling of Dangerous Substances/Preparation (67/548/EEC & 1999/45/EC, as amended):
The following label information applies:

Caution: Substance not yet fully tested.

Symbol(s):	Xn - Harmful
Risk Phrases:	R10 Flammable. R65 Harmful: may cause lung damage if swallowed.
Safety phrases:	S23 Do not breathe spray. S24 Avoid contact with skin. S62 If swallowed, do not induce vomiting: seek medical advice immediately and show this container or label.

Additional Governmental Inventories

Australia - Inventory of Chemical Substances (AICS): Not determined.

China: Not determined.

Japan - Existing and New Chemical Substances (ENCS): Not determined.

Korea - Existing and Evaluated Chemical Substances (ECL): Not determined.

Philippines - Inventory of Chemicals and Chemical Substances (PICCS): Not determined.

16. OTHER INFORMATION

Summary of changes:	Section 15
Supersedes:	August 2008
Prepared by:	UOP Health, Safety & Environmental Department

HMIS™ - Hazardous Material Identification System:

HMIS™ Ratings: 0-minimal hazard, 1- slight hazard, 2- moderate hazard, 3- serious hazard, 4- severe hazard.

HEALTH :	**2**
FLAMMABILITY :	**2**
REACTIVITY :	**0**

For additional information concerning this product, contact the following:

For health, safety and environmental information, please contact:

Product Stewardship Manager
UOP LLC
25 E. Algonquin Road
Des Plaines, IL 60017-5017
USA
Tel: +1-847-391-3189
Fax: +1-847-391-2953

Product Safety Steward Europe
UOP N.V.
Noorderlaan 147
B-2030 Antwerpen
Belgium
Tel: +32-3-5409-971
Fax: +32-3-5417-806

For technical or purchasing information, please contact:

Renewable Energy and Chemicals
UOP LLC
25 E. Algonquin Road
Des Plaines, IL 60017-5017 USA
Tel: +1-847-391-2789
Fax: +1-847-391-2253

PRODUCT EMERGENCIES

If you have a product-related emergency, resulting in an incident such as a spill or release of product or human exposure and need assistance from UOP, please contact the following number:

24-Hour EMERGENCY NUMBER (UOP LLC) : + 1 - 847 - 391 - 2123

WPAFB HAZARDOUS AGENT SUMMARY FORM

If the proposed study will involve the use of hazardous or potentially hazardous agents, please provide the following information. This form must be filled out for each chemical used in a study. Material Data Safety Sheets (MSDS), (if available) must be attached to each Hazardous Agent Summary Form, (HASF).

Chemical Name:(common Name) Cyclophosphamide

Estimated highest dose level 40 mg/kg

(Exposure Concentration): 4 mg/ml

Route(s) of administration: intraperitoneal (IP)

Duration of treatment: single injection

Estimated maximum length of time animals will be monitored following exposure: 24 hours

Estimated exposure time for personnel: no personnel exposure is expected

Estimated exposure concentration for personnel: no personnel exposure is expected

The following personal protective equipment (PPE) must be worn/used in the animal rooms. PPE must be removed before leaving the animal rooms.

- □ Disposable gowns
- √ Lab coats
- √ Disposable gloves (type) Nitrile
- √ Safety glasses (type) _____
- □ Goggles (type) _____
- □ Respirator (type) _____
- □ Scrubs
- □ Face Shields
- √ Shoe Covers
- □ Bouffant Cap
- □ Waterproof Boots
- □ Others _____

PI will inform personnel on proper processes, procedure and equipment needed to work safely with this chemical. Provide information below:

CP is a white powder. The CP dosing solution will be prepared by dissolving in saline. Preparation will be done in a laboratory fume hood. A labcoat, disposable gloves (chemically resistant), and safety glasses should be worn during preparation. Rats will be injected IP. Similar PPE should be worn during dosing procedure.

Health risks associated with this chemical: Carcinogen, teratogen
Exposure routes: inhalation, dermal, ingestion
Other controls: _____

Principal Investigator:

Signature William R. Howard Print Name William R. Howard Date 2/10/11

Environmental, Safety Officer, Air force:

Signature Kathy Kincaid Print Name KATHY KINCAID Date 2/10/11

Environmental, Safety Officer, Navy:

Signature Keith E. Johnson Print Name KEITH E. JOHNSON Date 10 FEB 2011

Material Safety Data Sheet

Version 4.0
Revision Date 03/13/2010
Print Date 01/27/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Cyclophosphamide monohydrate
Product Number	:	C7397
Brand	:	Sigma
Company	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+18003255832
Fax	:	+18003255052
Emergency Phone #	:	(314) 776-6555

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
Carcinogen, Target Organ Effect, Toxic by ingestion, Teratogen

Target Organs

Bone marrow, Bladder

GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)

H301	Toxic if swallowed.
H350	May cause cancer.

Precautionary statement(s)

P201	Obtain special instructions before use.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P308 + P313	IF exposed or concerned: Get medical advice/attention.

HMIS Classification

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	1
Physical hazards:	0

NFPA Rating

Health hazard:	2
Fire:	1
Reactivity Hazard:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	Toxic if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms : 2-[Bis(2-chloroethyl)amino]tetrahydro-2*H*-1,3,2-oxazaphosphorine 2-oxide
Cytoxan

Formula : $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$
Molecular Weight : 279.1 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Cyclophosphamide monohydrate			
6055-19-2	200-015-4	-	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing give artificial respiration Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Use personal protective equipment. Avoid dust formation. Avoid breathing dust. Ensure adequate ventilation. Evacuate personnel to safe areas.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: 2 - 8 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves.

Eye protection
Face shield and safety glasses

Skin and body protection
Choose body protection according to the amount and concentration of the dangerous substance at the work place.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	crystalline
Colour	white

Safety data

pH	no data available
Melting point	49 - 51 °C (120 - 124 °F) - lit.
Boiling point	no data available
Flash point	113 °C (235 °F) - closed cup
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents, Strong acids, Strong bases

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Oxides of phosphorus, Hydrogen chloride gas

11. TOXICOLOGICAL INFORMATION

Acute toxicity
LD50 Oral - rat - 94 mg/kg
Remarks: Behavioral:Ataxia. Kidney, Ureter, Bladder:Urine volume increased. Blood: Hemorrhage.

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
May alter genetic material.

Carcinogenicity

This is or contains a component that has been reported to be carcinogenic based on its IARC, OSHA, ACGIH, NTP, or EPA classification.

Possible human carcinogen

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	Known to be human carcinogen (Cyclophosphamide monohydrate)
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
May cause congenital malformation in the fetus.

Specific target organ toxicity - single exposure (GHS)
no data available

Specific target organ toxicity - repeated exposure (GHS)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	Toxic if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Additional Information
RTECS: RP6157750

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Observe all federal, state, and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 3464 Class: 6.1 Packing group: III
Proper shipping name: Organophosphorus compound, toxic, solid, n.o.s. (Cyclophosphamide monohydrate)
Reportable Quantity (RQ): 10 lbs
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 3464 Class: 6.1 Packing group: III EMS-No: F-A, S-A
Proper shipping name: ORGANOPHOSPHORUS COMPOUND, TOXIC, SOLID, N.O.S. (Cyclophosphamide monohydrate)
Marine pollutant: No

IATA
UN-Number: 3464 Class: 6.1 Packing group: III
Proper shipping name: Organophosphorus compound, toxic, solid, n.o.s. (Cyclophosphamide monohydrate)

15. REGULATORY INFORMATION

OSHA Hazards
Carcinogen, Target Organ Effect, Toxic by ingestion, Teratogen

DSL Status
All components of this product are on the Canadian DSL list.

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components

	CAS-No.	Revision Date
Cyclophosphamide monohydrate	6055-19-2	1993-04-24

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Cyclophosphamide monohydrate	6055-19-2	1993-04-24

New Jersey Right To Know Components

	CAS-No.	Revision Date
Cyclophosphamide monohydrate	6055-19-2	1993-04-24

California Prop. 65 Components

	CAS-No.	Revision Date
WARNING! This product contains a chemical known to the State of California to cause cancer. Cyclophosphamide monohydrate	6055-19-2	1992-11-09

California Prop. 65 Components

WARNING! This product contains a chemical known to the State of California to cause birth defects or other reproductive harm. Cyclophosphamide monohydrate	CAS-No. 6055-19-2	Revision Date 1992-11-09

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

APPENDIX B. INHALATION EXPOSURE SUMMARY REPORT: 90-DAY INHALATION EXPOSURE TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

Author

James E. Reboulet, MS

Brian A. Wong, Ph.D.
Senior Inhalation Toxicologist

Performing Laboratory

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2729 R St., Bldg 837, Area B
Wright-Patterson AFB, OH 45433-5707

Study Sponsor

U. S. Air Force, AFMC, Alternative Fuels Certification Office, ASC/WNN

REPORT PREPARATION

Report prepared by:
Brian A. Wong, Ph. D.,
Senior Inhalation Toxicologist

KEY PERSONNEL

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2729 R Street, Building 837, Area B
Wright Patterson AFB OH 45433

Co-Investigator:
Brian A. Wong, Ph.D.
Senior Inhalation Toxicologist

Staff:
James E. Reboulet, MS
Michael Grimm, BS

INTRODUCTION

The Office of the Secretary of Defense Assured Fuels Initiative is pursuing domestically produced alternative fuels for military use to decrease dependence on petroleum-based oil sources. An alternative fuel being evaluated is the biofuel, hydro-treated renewable jet (HRJ) fuel that is based on oils extracted from the camelina plant (Camelina sativa). Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of HRJ fuel by inhalation is needed to assess the risk of replacing or augmenting JP-8 by HRJ fuel. HRJ fuel has the potential for both vapor and aerosol (fuel droplets) exposures. The objective of this study is to assess the potential inhalation toxicity of a test substance when administered via inhalation exposure to Fischer 344 rats on a repeated basis for 90 days (5 days per week over 13 weeks, 65 total exposures).

STUDY DESIGN

This study had 4 target exposure concentrations, 0, 200, 700, and 2000 mg/m^3 of HRJ fuel, with 10 males and 10 female rats were in each exposure group. Each exposure group had two replicates, both with 5 males and 5 females. In order to accommodate the necropsy at the end of exposures, the replicates were staggered by one day in the exposure schedule. Due to the stagger in exposures, accommodation of holidays, and the neurobehavioral test schedule, the study spanned a total of 106 days from first exposure to last exposure. Each replicate had a total of 71 exposure days in that span.

MATERIALS AND METHODS

Test Material

The HRJ Fuel was obtained from the manufacturer (UOP, LLC, Des Plaines, IL) by the Fuels Branch at Wright Patterson Air Force Base (WPAFB). An additive package consisting of chemicals normally added to JP-8 jet fuel was added to the HRJ fuel by the Fuels Branch. The HRJ fuel with additives was labeled POSF 6152. Physical properties of HRJ fuel are listed in Table 1. The HRJ fuel in a five gallon drum was shipped by the sponsor to NAMRU-Dayton and stored in a well-ventilated area at room temperature. The method of synthesis of the HRJ fuel is being kept by the manufacturer. Information regarding composition and purity of the jet fuel and the additive package are maintained by the sponsor. A total of two drums were received over the course of this study. The material was used undiluted.

Exposure System

Rats were exposed by inhalation in a glass and stainless steel Toxic Hazard Research Unit (THRU) chamber with a volume of 690 L. Four chambers were used, one for each exposure group. The chambers have a capacity of up to 32 rats held in THRU stainless steel wire mesh cage units. The THRU exposure chambers were operated at total flow rate of approximately 180 L/min to provide at least one complete air change in 3.8 minutes (15.8 air changes/hour; minimum guideline requirements are 10 air changes per hour) and a T_{99} equilibrium time of approximately 18 minutes (T_{99} is the time for the concentration of test substance in the chamber

to rise from background or zero to 99% of the equilibrium or target concentration). This chamber size and airflow rate was considered adequate to maintain an oxygen level that is at least 19%, the minimum required by the guidelines. At the end of an exposure, the chamber was operated at approximately the same flow rate using clean air for approximately 30 minutes so that the bulk of the test material was cleared from the chamber before removing the animals.
The chambers were located in room 264 of Building 837 which was supplied with air that had passed through a 95% HEPA filter. The filtered room air was distributed by a blower to the exposure chambers. Air flow was measured by monitoring the pressure drop across a laminar flow element at the inlet to each chamber. The temperature in each chamber was monitored using a type J thermocouple. Relative humidity in the control chamber was measured by using a humidity temperature transmitter (Omega Engineering, Inc.). As the chambers are supplied by a common blower, the relative humidity in the other chambers was approximately the same as in the control chamber. Air leaving the chambers was pulled by a blower to the building exhaust flow system.

Chamber distribution

There are nine sampling ports on the back of the Toxic Hazard Research Chamber (THRU). To check the uniformity of distribution, the ports were equipped with sampling lines which reached to the midline of the chamber. All four cages were in place during the chamber distribution test. The generation system was operated to achieve the approximate target concentration. Ten minute samples were taken from the center port, then an alternate port, and again from the center port. During the second sampling from the center port another of the alternate ports was connected and sampling continued in this fashion until the 8 alternate ports were sampled.

Generation System

The jet fuel was generated as a mixture of aerosol and vapor by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton, IL). A liquid metering pump (FMI, Fluid Metering, Inc., Syosset, NY) pumped liquid jet fuel from a glass bottle reservoir to the nozzle. Compressed instrument air at approximately 50 psi was supplied to the nozzle. The spray was directed into a custom-made PVC mixing volume and then injected into the inlet air stream to the exposure chamber.

HRJ fuel Concentration Measurement: A fourier transform infrared spectrophotometer (FTIR, Model 380, Thermo Scientific, Waltham MA) was used to monitor the concentration of jet fuel in the chamber. A sample of the chamber atmosphere was pulled through the FTIR spectrophotometer. The signal output from the FTIR was recorded by computer. FTIR with serial number (SN) ECN089868 was connected to the Control Chamber, FTIR with SN ECN086884 was connected to the Low Chamber (200 mg/m^3), FTIR with SN ECN092402 was connected to the Medium Chamber (700 mg/m^3), and FTIR with SN ECN089069 was connected to the High Chamber (2000 mg/m^3).

FTIR Calibration: The FTIR was calibrated using standard bag methodology. Tedlar bags containing known volumes of air were prepared. The bags were injected with a volume of HRJ fuel to yield the desired concentrations. After injection the bag was warmed with a hot air gun to

assure that the HRJ was totally volatilized. The bags were allowed to cool for approximately 5 minutes to ambient temperature, which established equilibrium between the ambient aerosol and vapor components. The bags were agitated to assure thorough mixing and FTIR analysis was performed using a filtered 10 cm glass gas cell, at a flow rate of 350 ml per minute. This procedure was replicated between at least 2 times for each concentration.

FTIR absorption measurements were recorded approximately every 20 seconds until the maximum absorption reading was reached and maintained for several minutes. Each recording was saved as a line in a text file, which was subsequently imported into Excel for plotting. A calibration curve of spectrophotometer response as a function of jet fuel concentration in milligrams per meter cubed (mg/m^3) was produced and the curve input into the FTIR computer.

Nominal concentration was calculated from the air flow rate through the chamber, the total generation time and the mass of fuel consumed during the exposure. The reservoirs containing the jet fuel used by the generation system were weighed at the start of generation and at the end, after the exposure period. A flow rate of 180 L/min and exposure time of 360 minutes were used in the calculation.

RESULTS

A protocol deviation was documented in which the intermediate and high concentration groups of animals were inadvertently switched when placed in the exposure chambers on study day 48, June 27, 2011. Hence, the intermediate group was exposed to the high concentration (2000 mg/m^3) for one day, and the high concentration group was exposed to the intermediate concentration (700 mg/m^3) for one day. The control and low exposure group were unaffected. After the one day switch, animals were placed in the proper chambers for the remaining exposures. The exposure concentrations were recalculated with one day's concentration switched between the intermediate and high exposure group (Table 3b). Based on the overall results and observations from the study, there were no effects that could be attributed to the switched exposures.

Test Substance Characterization

The HRJ fuel was used as supplied by the sponsor and the Fuels Branch, WPAFB, Dayton, OH. Information and data regarding the manufacture, composition, physical and chemical characteristics may be obtained from the Fuels Branch, WPAFB through the sponsor.

Exposure period: For this study, the exposure period started when the compressed air and the HRJ fuel flow were applied to the nozzle. The concentration in the chamber began to increase immediately, as observed on the FTIR. At the end of the exposure period, the compressed air and fuel flow to the nozzle were shut off. Animals were maintained in the chambers with a continuous flow of clean air for approximately 30 minutes. Animals were moved from the exposure chamber to domiciliary housing in the WPAFB vivarium. Control animals were housed in a separate room in the WPAFB vivarium.

Chamber Distribution: The chamber distribution was checked in the high concentration chamber prior to the start of exposures. The measured concentration at the standard sampling

location at the center of the chamber provided an indication of the variability of concentration over time, while the measurements at the surrounding ports provided an indication of the variability of concentration on a spatial basis. The chamber distribution test results indicate that the variability of concentration in the chamber is less than 2% (Table 2). To further minimize any effects due to variability of concentration within the chamber, animals were placed in different cage locations over the course of the experiment.

Exposure Conditions: Over the course of the exposures, concentration, temperature, humidity, air flow, and static pressure readings were monitored (Table 3). The humidity and air flow remained at targets, and did not deviate outside of prescribed ranges. The chamber temperatures were recorded three times per exposure period (after initially being recorded twice per day at the beginning of the study). The study average temperatures were 22.1±0.7, 22.6±0.7, 22.7±0.6, 22.3±0.7 °C, for the 0, 200, 700, and 2000 mg/m^3 chambers, respectively. The HRJ fuel concentration in a chamber was measured continuously by FTIR, and an exposure period average recorded at the end of each day's exposure. The study averages of daily chamber concentrations were 0.9±2.4, 194.8±15.3, 702.5±29.8, and 1990.5±52.4 mg/m^3 for the 0, 200, 700, and 2000 mg/m^3 chambers, respectively. Nominal concentrations, based on the HRJ fuel used and the chamber air flow were 245.9 ± 50.2, 816.5 ± 114.4 and 2411.4 ± 170.2 mg/m^3, giving analytical to nominal concentration ratios of 0.82, .87, and 0.83, respectively (Table 3).

The average exposure concentrations to which animals were exposed (Table 3) were different from the measured chamber concentrations due to an inadvertent switch of animals between the intermediate and high concentration chambers for one exposure day (Table 3b). The resulting overall average concentration for the intermediate group was higher by approximately 2.5% than the measured concentration in the intermediate chamber and 2.8% higher than the target concentration (Table 3b), while the high concentration group was 1% lower than the measured concentration in the high chamber and lower by 1.3% from the target (Table 3b). The effect of one day's switch in exposure concentration on the overall average exposure concentration was minimal (less than 2.8% or 1.3% difference from target). And, as the switch took place in the middle of the exposure schedule, the animals had several weeks of exposure to the appropriate concentrations before any biological assessments were made. Thus, the switch of intermediate and high group animals for one day was considered to have no impact on the results of the study.

The aerosol mass concentration was measured using gravimetric filters. The average aerosol concentrations were 4.6 ± 3.0 and 242.7 ± 34.6 mg/m^3 for the intermediate and high exposure concentration chambers, respectively (Table 4). There were some problems with equipment measuring the aerosol in the intermediate concentration chamber during weeks 11-15 towards the end of exposures. No aerosol was detected in the low concentration chamber. The aerosol fraction was 0.7% and 12% of the total jet fuel concentration in the intermediate and high concentration chambers, respectively. Thus, as the total HRJ fuel concentration increased the fraction of the total that existed as aerosol droplets increased.

A cascade impactor (In-Tox products, Moriarty, NM) was used to measure the particle size distribution. Measurements were made by sampling from a chamber approximately once during a week. The average mass median aerodynamic diameter and geometric standard deviation (MMAD (GSD)) of the aerosols were calculated as 3.06 (2.06) and 2.60 (1.64) µm for the

intermediate and high concentration chambers, respectively (Table 5). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents.

Daily averages for overall HRJ fuel concentration and nominal concentration are presented in Table 6. The fuel consumed during each day's exposure for the nominal concentration is presented in Table 7. The measured chamber temperatures in the Control and Low (200 mg/m^3) Concentration chambers are listed in Table 8. The measured chamber temperatures in the Medium (700 mg/m^3) and High Concentration (2000 mg/m^3) chambers are listed in Table 9.

CONCLUSION

The concentration of HRJ Fuel in the exposure chambers was monitored using fourier transform infrared spectrophotometry and was measured approximately continuously during each exposure period. The HRJ Fuel exposure was conducted for 6 hours/day, 5 days/week for more than13 weeks. The average daily mean ± standard deviations were 0.9±2.4, 194.8±15.3, 719.8±154.2, and 1973.2±156.4 mg/m^3 for the target concentrations of 0, 200,700, and 2000 mg/m^3 HRJ Fuel in air, respectively. A deviation in the protocol that caused the switch of intermediate and high group animals for one day had no impact on the results of the study.

The environmental parameters specified in the protocol for temperature, relative humidity and airflow were maintained at or near the target set points of 72 ºF, 50% and 180 L/min, respectively, throughout the entire study. The exposures were performed from April 28, 2011 through July 31, 2011.

Table 1. Test Material

Name:	**Hydrotreated Renewable Jet (HRJ) Fuel with additives: HRJ Camelina**
	Bio-oil Derived SPK
CAS #:	NA
Formula:	C_9-C_{15} paraffin bio-oil derived
Molecular Weight	NA, mixture
Description:	Colorless liquid
Test Substance Category:	Fuels. Jet Aircraft. OSHA combustible liquid.
Storage:	Keep container closed tightly in cool, well-ventilated place. Keep away from heat, and sources of ignition.
Stability:	Stable at normal conditions. Decomposes upon heating.
Supplier:	UOP through AFRL Fuels Branch
Lot Number:	POSF 6152

Expiration date: One year from date of receipt.

Table 2. Chamber Uniformity Measurements

Position	Avg conc (mg/m^3)	Position	Avg Conc (mg/m^3)
Center	1983.06	Top left	1992.46
Center	1991.16	Top Middle	2010.22
Center	1991.16	Center Left	2024.94
Center	1963.43	Bottom Left	2033.62
Center	1963.43	Bottom Middle	2007.94
Center	2031.22		
Center	2031.22	Bottom Right	2004.02
Center	2022.97		
Center	2022.97	Center Right	2008.45
Center	2053.32		
Center	2053.32	Top Right	2020.05
Center	2016.97		
		Center (average)	2010.35
Average	2010.35	Average	2012.45
Stdev	31.35	Stdev	12.14
Temporal CV	0.016	Total variation CV	0.006
Spatial CV	-0.0002		

$(CV_{Spatial})^2 = (CV_{Total})^2 - (CV_{Temporal})^2$ Ref: Cheng and Moss, 1985, Inhalation Exposure Systems, in Concepts in inhalation Toxicology, 2nd ed., R. O McClellan and R. F. Henderson, eds., Taylor and Francis, Washington, DC.

Table 3. Chamber atmosphere summary

	Target Concentration	0 (mg/m^3)	200 (mg/m^3)	700 (mg/m^3)	2000 (mg/m^3)
Chamber Temperature °C	Mean	**22.1**	**22.6**	**22.7**	**22.3**
	St. Dev	0.7	0.7	0.6	0.7
	N	73	73	73	73
Chamber Concentration (mg/m^3)	Mean	**0.9**	**194.8**	**719.8**	**1973.2**
	St. Dev	2.4	15.3	154.2	156.4
Gravimetric Concentration (mg/m^3)			NM	**4.63**	**242.7**
				2.96	34.6
Proportion of total Concentration				0.007	0.12
Particle Size	MMAD (μm)		NM	**3.06**	**2.60**
	GSD			2.06	1.64

NM = not measured. Aerosol was not detected in the low concentration chamber.

Table 3b. Chamber atmosphere summary with consideration of exposure switch

	Intermediate (700 mg/m^3)	High (2000 mg/m^3)
Chamber Concentration (Mean ± St. Dev)	702.5 ± 29.8	1990.5 ± 52.4
Group Concentration with switch[1]	719.8	1973.2
% Difference from Chamber Concentration	2.5%	-0.9%
% Difference from Target Concentration	2.8%	-1.3%

[1]Intermediate and high exposure groups were inadvertently loaded into the incorrect chambers. The average concentration experienced by the groups was recalculated to include the one day of the switched exposure concentration. These values are used for the exposure concentrations in Table 3.

Table 4. Aerosol Concentration Measurements

Week	Aerosol Concentration High Chamber mg/m^3	Medium Chamber mg/m^3
1	215.0	5.5
2	235.4	6.1
3	250.5	1.3
4	246.7	
5	222.7	7.7
6	226.7	3.9
7	205.4	7.1
8	220.1	9.3
9	226.9	0.7
10	221.8	1.6
11	225.5	
12	298.9	
13	290.6	
14	324.5	
15		
16	229.3	3.1
Average	242.7	4.6
SD	34.6	3.0

SD = standard deviation

Table 5. Particle Size Distribution Results

	High Chamber		Medium Chamber	
Week	**MMAD**	**sigma g**	**MMAD**	**sigma g**
1	2.65	1.5	2.51	1.65
2	2.66	1.6	3.16	1.71
3	2.65	1.46	2.85	1.72
4	2.79	1.72	3.64	1.62
5	2.49	1.61	3.84	2.37
6	2.62	1.63	3.11	2.04
7	2.56	1.62	2.73	2.51
8	2.36	1.58	2.34	2.61
9	2.78	1.78		
10	2.24	1.62		
11	2.5	1.82		
12	2.64	1.68		
13	2.73	1.66		
14	2.71	1.63		
15				
16	2.58	1.66	3.39	2.32
	MMAD	sigma g	MMAD	sigma g
Average	2.60	1.64	3.06	2.06
SD	0.15	0.09	0.50	0.40

Table 6. Daily Measured and Nominal HRJ Concentrations

Study Day		Control	Low (200 mg/m^3)			Medium (700 mg/m^3)			High (2000 mg/m^3)		
		mg/M^3	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal
1		1.1	168.6	231.5	72.8	707.6	756.2	93.6	2018.9	2250.0	89.7
2		0.6	196.4	222.2	88.4	698.9	759.3	92.1	2038.7	2274.7	89.6
3		0.5	207.2	194.4	106.6	673.1	760.8	88.5	1977.5	2216.0	89.2
4	Sat										
5	Sun										
6		0.3	204.2	216.0	94.5	714.6	652.8	109.5	1993.4	2273.1	87.7
7		0.3	188.1	288.6	65.2	711.9	787.0	90.5	2020.0	2302.5	87.7
8		0.9	190.1	402.8	47.2	712.4	759.3	93.8	2012.6	2299.4	87.5
9		0.9	186.9	256.2	72.9	714.8	771.6	92.6	2016.9	2324.1	86.8
10		0.1	198.4	260.8	76.1	726.5	779.3	93.2	2018.7	2327.2	86.7
11	Sat										
12	Sun										
13		0.6	194.3	256.2	75.9	721.9	767.0	94.1	2031.7	2317.9	87.7
14		-0.6	184.6	251.5	73.4	712.9	774.7	92.0	1986.0	2341.0	84.8
15		1.3	196.4	248.5	79.1	713.5	767.0	93.0	1986.3	2373.5	83.7
16		1.6	193.3	253.1	76.4	696.5	703.7	99.0	2006.0	2240.7	89.5
17			185.5	324.1	57.2	700.7	743.8	94.2	2013.3	2259.3	89.1
18	Sat										
19	Sun										
20	Holiday										
21		-0.1	189.3	262.3	72.1	712.0	757.7	94.0	2022.2	2294.8	88.1
22		1.1	187.3	262.3	71.4	713.2	739.2	96.5	2005.1	2302.5	87.1
23		0.4	183.3	262.3	69.9	679.8	779.3	87.2	2033.2	2308.6	88.1
24		0.6	195.2	262.3	74.4	698.0	756.2	92.3	2002.9	2267.0	88.4
25	Sat										
26	Sun										

Table 6. Daily Measured and Nominal HRJ Concentrations (continued)

Study Day		Control	Low (200 mg/m^3)			Medium (700 mg/m^3)			High (2000 mg/m^3)		
		mg/M^3	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal
28		0.6	194.5	253.1	76.8	704.1	754.6	93.3	2011.0	2293.2	87.7
29		0.5	212.4	296.3	71.7	705.6	751.5	93.9	1987.2	2280.9	87.1
30		0.4	206.5	280.9	73.5	703.0	763.9	92.0	2010.1	2216.0	90.7
31		0.2	188.8	296.3	63.7	683.1	768.5	88.9	2032.1	2299.4	88.4
32	Sat										
33	Sun										
34		0.8	201.7	279.3	72.2	715.2	745.4	96.0	2008.7	2285.5	87.9
35		1.0	199.4	263.9	75.6	714.5	767.0	93.2	2023.5	2231.5	90.7
36		0.1	201.3	415.1	48.5	710.0	754.6	94.1	2006.1	2277.8	88.1
37		0.3	208.1	213.0	97.7	709.9	745.4	95.2	2013.6	2270.1	88.7
38		0.6	209.5	225.3	93.0	727.9	760.8	95.7	2030.1	2285.5	88.8
39	Sat										
40	Sun										
41		0.7	211.1	219.1	96.3	719.3	759.3	94.7	2015.1	2284.0	88.2
42		0.2	189.8	216.0	87.8	718.8	756.2	95.1	2005.1	2276.2	88.1
43		0.1	196.9	202.2	97.4	715.4	784.0	91.3	2001.3	2305.6	86.8
44		0.5	199.5	217.6	91.7	718.2	737.7	97.4	1936.4	2344.1	82.6
45			188.9	205.2	92.0	694.4	767.0	90.5	1998.7	2327.2	85.9
46	Sat										
47	Sun										
48		0.5	201.1	208.3	96.5	723.1	760.8	95.0	2004.1	2316.4	86.5
49		5.5	199.3	206.8	96.4	716.6	762.3	94.0	2018.3	2336.4	86.4
50		0.4	198.3	219.1	90.5	717.7	765.4	93.8	1999.2	2296.3	87.1
51		-0.3	208.6	223.8	93.2	655.5	865.7	75.7	2023.8	2296.3	88.1
52		0.0	200.6	203.7	98.5	718.7	762.3	94.3	2030.7	2277.8	89.2
53	Sat										
54	Sun										
55	Holiday										

Table 6. Daily Measured and Nominal HRJ Concentrations (continued)

Study Day		Control	Low (200 mg/m^3)			Medium (700 mg/m^3)			High (2000 mg/m^3)		
		mg/M^3	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal	Measured mg/M^3	nominal mg/M^3	% of nominal
56		0.1	201.5	219.1	92.0	722.0	763.9	94.5	2023.9	2299.4	88.0
57		0.1	213.3	225.3	94.6	714.9	757.7	94.4	2012.4	2314.8	86.9
58		0.1	202.1	203.7	99.2	718.3	1547.8	46.4	1996.1	2314.8	86.2
59		-0.1	204.7	213.0	96.1	709.9	751.5	94.5	1977.0	2333.3	84.7
60	Sat										
61	Sun										
62		-0.2	207.2	209.9	98.7	703.6	765.4	91.9	1969.7	2314.8	85.1
63		-0.1	203.9	211.4	96.4	708.6	762.3	92.9	1993.5	2341.0	85.2
64		0.0	210.8	226.9	92.9	710.6	760.8	93.4	2007.9	2333.3	86.1
65		0.0	189.9	194.4	97.7	707.3	760.8	93.0	2003.3	2339.5	85.6
66		18.3	206.5	217.6	94.9	713.6	759.3	94.0	2010.6	2347.2	85.7
67	Sat										
68	Sun										
69		-0.2	191.4	216.0	88.6	698.8	745.4	93.8	1982.9	2310.2	85.8
70		-0.1	201.8	211.4	95.4	693.0	759.3	91.3	1998.5	2354.9	84.9
71		0.3	198.8	209.9	94.7	697.9	756.2	92.3	1978.1	2328.7	84.9
72		0.0	198.6	225.3	88.1	693.4	831.8	83.4	2048.9	2435.2	84.1
73		0.0	207.1	206.8	100.1	691.0	841.0	82.2	2004.6	2390.4	83.9
74	Sat										
75	Sun										
76		0.2	199.7	206.8	96.6	678.5	804.0	84.4	1811.3	2395.1	75.6
77		0.3	173.5	231.5	74.9	562.8	973.8	57.8	1660.4	2709.9	61.3
78		1.0	190.1	262.3	72.4	548.0	969.1	56.5	2004.5	2598.8	77.1
79		0.4	177.0	257.7	68.7	718.4	918.2	78.2	1921.5	2507.7	76.6
80		0.8	186.7	276.2	67.6	693.6	858.0	80.8	1938.7	2637.3	73.5
81	Sat										
82	Sun										

Table 6. Daily Measured and Nominal HRJ Concentrations (continued)

Study Day		Control mg/M³	Low (200 mg/m³) Measured mg/M³	Low (200 mg/m³) nominal mg/M³	Low (200 mg/m³) % of nominal	Medium (700 mg/m³) Measured mg/M³	Medium (700 mg/m³) nominal mg/M³	Medium (700 mg/m³) % of nominal	High (2000 mg/m³) Measured mg/M³	High (2000 mg/m³) nominal mg/M³	High (2000 mg/m³) % of nominal
83		0.5	148.2	314.8	47.1	717.8	898.1	79.9	1962.9	2631.2	74.6
84		0.0	158.9	478.4	33.2	723.2	907.4	79.7	1937.9	2683.6	72.2
85		7.6	180.1	277.8	64.8	731.4	930.6	78.6	1987.3	2682.1	74.1
86		2.6	216.3	277.8	77.9	718.7	925.9	77.6	1910.5	2709.9	70.5
87		0.6	210.3	243.8	86.3	713.1	913.6	78.1	1937.0	2722.2	71.2
88	Sat										
89	Sun										
90		0.6	210.2	282.4	74.4	721.6	941.4	76.7	1984.5	2708.3	73.3
91		1.1	218.8	268.5	81.5	722.3	936.7	77.1	1993.3	2720.7	73.3
92		1.1	122.1	299.4	40.8	692.6	941.4	73.6	1968.8	2669.8	73.7
93		0.8	201.0	185.2	108.5	637.3	963.0	66.2	2022.0	2700.6	74.9
94		1.5	183.5	205.2	89.4	710.4	927.5	76.6	2009.0	2634.3	76.3
95	Sat										
96	Sun										
97		0.2	197.4	231.5	85.3	661.4	959.9	68.9	1997.9	2642.0	75.6
98		0.9	194.6	222.2	87.6	696.7	921.3	75.6	2003.2	2682.1	74.7
99		0.6	190.9	222.2	85.9	721.1	862.7	83.6	1985.2	2642.0	75.1
100		0.8	199.9	206.8	96.7	716.2	854.9	83.8	1999.4	2652.8	75.4
101		0.8	196.8	222.2	88.6	708.6	862.7	82.1	1954.7	2575.6	75.9
102	Sat										
103	Sun										
104		0.7	196.4	226.9	86.6	719.5	831.8	86.5	1945.2	2623.5	74.1
105		0.6	188.4	222.2	84.8	717.7	848.8	84.6	2000.8	2688.3	74.4
106		0.2	202.0	213.0	94.8	700.7	834.9	83.9	2009.8	2731.5	73.6
Average		0.9	194.8	245.9	82.0	702.5	816.5	87.4	1990.5	2411.4	83.0
StDev		2.4	15.3	50.2	15.7	29.8	114.4	10.5	52.4	170.2	6.6

Table 7. Fuel Consumption for Determination of Nominal Concentration

Study	Low (200 mg/m^3)			Med (700 mg/m^3)			High (2000 mg/m^3)		
Day	Start	End	Nominal	Start	End	Nominal	start	end	nominal
1	1061.0	915.2	2250	550.6	501.6	756	512.0	497.0	231
2	915.2	767.8	2275	501.6	452.4	759	497.0	482.6	222
3	767.8	624.2	2216	452.4	403.1	761	482.6	470.0	194
4	sat								
5	sun								
6	999.8	852.5	2273	580.3	538.0	653	470.0	456.0	216
7	852.5	703.3	2302	538.0	487.0	787	456.0	437.3	289
8	1108.4	959.4	2299	487.0	437.8	759	604.1	578.0	403
9	959.4	808.8	2324	570.5	520.5	772	578.0	561.4	256
10	887.2	736.4	2327	598.7	548.2	779	638.8	621.9	261
11	sat								
12	sun								
13	1298.9	1148.7	2318	548.2	498.5	767	621.3	604.7	256
14	1148.3	996.6	2341	498.3	448.1	775	604.1	587.8	252
15	996.3	842.5	2373	761.4	711.7	767	588.2	572.1	248
16	842.2	697.0	2241	711.5	665.9	704	570.1	553.7	253
17	1264.2	1117.8	2259	665.6	617.4	744	553.2	532.2	324
18	sat								
19	sun								
20	Holiday								
21	1117.6	968.9	2295	617.6	568.5	758	536.5	519.5	262
22	968.9	819.7	2302	568.4	520.5	739	519.5	502.5	262
23	819.7	670.1	2309	520.5	470.0	779	502.5	485.5	262
24	1318.4	1171.5	2267	716.7	667.7	756	485.5	468.5	262
25	sat								
26	sun								
27	1168.6	1021.7	2267	667.6	620.5	727	468.4	450.7	273
28	1021.6	873.0	2293	620.5	571.6	755	735.8	719.4	253
29	873.4	725.6	2281	571.8	523.1	752	718.4	699.2	296
30	1248.4	1104.8	2216	712.1	662.6	764	699.2	681.0	281
31	1101.2	952.2	2299	662.0	612.2	769	681.2	662.0	296
32	sat								
33	sun								

Table 7. Fuel Consumption for Determination of Nominal Concentration (continued)

Study	Low (200 mg/m^3)			Med (700 mg/m^3)			High (2000 mg/m^3)		
Day	start	end	nominal	start	end	nominal	Start	end	nominal
34	952.6	804.5	2285	612.0	563.7	745	662.1	644.0	279
35	802.5	657.9	2231	563.3	513.6	767	643.5	626.4	264
36	1172.1	1024.5	2278	716.3	667.4	755	626.6	599.7	415
37	1024.2	877.1	2270	667.4	619.1	745	599.7	585.9	213
38	877.1	729.0	2285	619.1	569.8	761	586.0	571.4	225
39	sat								
40	sun								
41	1294.3	1146.3	2284	569.7	520.5	759	571.4	557.2	219
42	1146.4	998.9	2276	520.5	471.5	756	557.3	543.3	216
43	998.9	849.5	2306	695.8	645.0	784	542.8	529.7	202
44	849.5	697.6	2344	645.2	597.4	738	529.8	515.7	218
45	1312.9	1162.1	2327	597.2	547.5	767	515.9	502.6	205
46	sat								
47	sun								
48	1161.8	1011.7	2316	547.5	498.2	761	502.6	489.1	208
49	1011.5	860.1	2336	498.2	448.8	762	489.3	475.9	207
50	860.3	711.5	2296	712.6	663.0	765	475.9	461.7	219
51	1289.3	1140.5	2296	663.2	607.1	866	760.5	746.0	224
52	1140.6	993.0	2278	606.9	557.5	762	746.7	733.5	204
53	sat								
54	sun								
55	Holiday								
56	992.6	843.6	2299	557.0	507.5	764	732.6	718.4	219
57	843.5	693.5	2315	506.5	457.4	758	718.7	704.1	225
58	1303.5	1153.5	2315	773.5	673.2	1548	704.2	691.0	204
59	1153.3	1002.1	2333	673.3	624.6	752	691.1	677.3	213
60	sat								
61	sun								
62	1002.2	852.2	2315	624.8	575.2	765	677.3	663.7	210
63	852.2	700.5	2341	575.3	525.9	762	663.4	649.7	211
64	1239.3	1088.1	2333	526.0	476.7	761	649.8	635.1	227
65	1087.9	936.3	2340	696.3	647.0	761	634.7	622.1	194
66	936.3	784.2	2347	647.2	598.0	759	622.3	608.2	218
67	sat								
68	sun								

Table 7. Fuel Consumption for Determination of Nominal Concentration (continued)

Study	Low (200 mg/m^3)			Med (700 mg/m^3)			High (2000 mg/m^3)		
Day	start	end	nominal	start	end	nominal	Start	end	nominal
69	784.2	634.5	2310	598.1	549.8	745	608.3	594.3	216
70	1248.3	1095.7	2355	549.9	500.7	759	594.5	580.8	211
71	1095.7	944.8	2329	500.9	451.9	756	580.9	567.3	210
72	944.7	786.9	2435	735.5	681.6	832	567.5	552.9	225
73	787.1	632.2	2390	681.6	627.1	841	552.9	539.5	207
74	sat								
75	sun								
76	1269.8	1114.6	2395	627.2	575.1	804	539.6	526.2	207
77	1115.1	939.5	2710	575.0	511.9	974	526.5	511.5	231
78	940.9	772.5	2599	510.9	448.1	969	511.5	494.5	262
79	1323.0	1160.5	2508	733.7	674.2	918	494.3	477.6	258
80	1162.4	991.5	2637	674.6	619.0	858	476.3	458.4	276
81	sat								
82	sun								
83	985.4	814.9	2631	619.2	561.0	898	457.5	437.1	315
84	814.8	640.9	2684	561.7	502.9	907	773.2	742.2	478
85	1232.5	1058.7	2682	503.2	442.9	931	742.6	724.6	278
86	1059.4	883.8	2710	759.8	699.8	926	724.2	706.2	278
87	883.8	707.4	2722	700.1	640.9	914	706.5	690.7	244
88	sat								
89	sun								
90	1264.2	1088.7	2708	642.1	581.1	941	691.0	672.7	282
91	1089.3	913.0	2721	581.8	521.1	937	672.8	655.4	269
92	910.6	737.6	2670	521.8	460.8	941	654.7	635.3	299
93	1185.1	1010.1	2701	739.4	677.0	963	634.8	622.8	185
94	1010.0	839.3	2634	677.5	617.4	927	622.1	608.8	205
95	sat								
96	sun								
97	839.2	668.0	2642	619.8	557.6	960	609.5	594.5	231
98	1290.2	1116.4	2682	557.9	498.2	921	594.5	580.1	222
99	1116.5	945.3	2642	498.5	442.6	863	580.1	565.7	222
100	945.2	773.3	2653	768.6	713.2	855	565.8	552.4	207
101	773.3	606.4	2576	714.2	658.3	863	551.6	537.2	222
102	sat								
103	sun								
104	1290.1	1120.1	2623	659.2	605.3	832	537.4	522.7	227
105	1119.8	945.6	2688	605.9	550.9	849	522.9	508.5	222
106	945.4	768.4	2731	551.2	497.1	835	508.7	494.9	213

Nominal = mass of fuel used / (airflow * exposure time); (start (g) – end (g))/64.8 m^3

Table 8. Chamber Temperature in the Control and Low Concentration Chambers

Study	Control			Daily		Low (200 mg/m^3)			Daily	
Day	AM	mid	PM	Avg	St.dev	AM	Mid	PM	Avg	St.dev
1	21.1		21.5	21.3	0.3	21.8		22.4	22.1	0.4
2	21.8		22.1	22.0	0.2	22.3		22.8	22.6	0.4
3	21.3		22.3	21.8	0.7	21.8		22.9	22.4	0.8
4										
5										
6	21.6		22.0	21.8	0.3	22.4			22.4	
7	21.2		22.1	21.7	0.6	22.2		22.8	22.5	0.4
8	21.4		22.0	21.7	0.4	22.3		23.0	22.7	0.5
9	21.8		22.2	22.0	0.3	22.4		23.1	22.8	0.5
10	21.4			21.4		22.2		23.0	22.6	0.6
11										
12										
13	21.3		22.1	21.7	0.6	22.0		23.2	22.6	0.8
14	19.1		20.9	20.0	1.3	20.1		21.9	21.0	1.3
15	18.8		20.4	19.6	1.1	19.8		21.7	20.8	1.3
16			22.7	22.7				23.4	23.4	
17	21.9		22.2	22.1	0.2	22.7		23.1	22.9	0.3
18										
19										
20										
21	21.7		22.1	21.9	0.3	22.5		23.1	22.8	0.4
22	21.6		22.2	21.9	0.4	22.1		23.1	22.6	0.7
23	21.3		22.6	22.0	0.9	22.0		23.1	22.6	0.8
24	22.1		22.2	22.2	0.1	22.8		23.1	23.0	0.2
25										
26										
27	21.3		22.1	21.7	0.6	21.7		23.1	22.4	1.0
28	21.2		22.5	21.9	0.9	21.8		23.2	22.5	1.0
29			22.6	22.6				23.2	23.2	
30	21.6		22.4	22.0	0.6	22.0		23.1	22.6	0.8
31	21.4		22.3	21.9	0.6	21.9		23.1	22.5	0.8
32										
33										
34	21.6		23.4	22.5	1.3	21.9		23.1	22.5	0.8
35	21.4		22.4	21.9	0.7	22.2		23.2	22.7	0.7
36	22.1		22.3	22.2	0.1	22.8		23.1	23.0	0.2
37	21.6		22.1	21.9	0.4	22.5		23.1	22.8	0.4
38										
39										
40										

Table 8. Chamber Temperature in the Control and Low Concentration Chambers (continued)

Study	Control			Daily		Low (200 mg/m^3)			Daily	
Day	AM	mid	PM	Avg	St.dev	AM	Mid	PM	Avg	St.dev
41	21.5		22.8	22.2	0.9	21.9		23.2	22.6	0.9
42	22.1		22.4	22.3	0.2	20.9		23.1	22.0	1.6
43	22.1		22.4	22.3	0.2	22.6		23.1	22.9	0.4
44	20.8		22.4	21.6	1.1	21.4		23.2	22.3	1.3
45	22.4		22.7	22.6	0.2	22.7		23.3	23.0	0.4
46										
47										
48	21.6		22.7	22.2	0.8	22.1		23.2	22.7	0.8
49	21.2		22.6	21.9	1.0	21.6		23.1	22.4	1.1
50	21.8		22.7	22.3	0.6	21.9		23.4	22.7	1.1
51	20.9	22.1	22.4	21.8	0.8	21.5	22.6	23.0	22.4	0.8
52	21.8	22.4	22.6	22.3	0.4	22.1	23.0	23.1	22.7	0.6
53										
54										
55										
56	21.1	22.6	22.6	22.1	0.9	21.5	23.2	23.3	22.7	1.0
57	21.6	22.8	22.6	22.3	0.6	22.3	23.1	22.8	22.7	0.4
58	21.4	22.5	22.7	22.2	0.7	21.7	22.8	23.1	22.5	0.7
59	21.4	22.7	23.0	22.4	0.9	21.4	22.7	23.1	22.4	0.9
60										
61										
62	22.1	22.4	22.9	22.5	0.4	22.2	22.8	23.1	22.7	0.5
63	21.1	22.0	22.4	21.8	0.7	21.7	22.8	23.1	22.5	0.7
64	21.6	23.1	23.1	22.6	0.9	21.3	23.3	23.4	22.7	1.2
65	21.6	22.7	23.1	22.5	0.8	21.9	23.6	23.6	23.0	1.0
66	22.1	23.0	23.6	22.9	0.8	21.9	23.3	23.8	23.0	1.0
67										
68										
69	23.3	24.3	23.8	23.8	0.5	23.1	23.5	23.7	23.4	0.3
70	22.2	23.3	23.1	22.9	0.6	22.4	23.7	23.6	23.2	0.7
71	22.1	23.1	23.4	22.9	0.7	22.4	23.2	23.6	23.1	0.6
72	22.0	23.3	23.6	23.0	0.9	22.6	23.7	23.2	23.2	0.6
73	21.8	23.4	23.1	22.8	0.9	21.9	23.6	23.4	23.0	0.9
74										
75										

Table 8. Chamber Temperature in the Control and Low Concentration Chambers (continued)

Study Day	AM	mid	PM	Daily Avg	St.dev	AM	Mid	PM	Daily Avg	St.dev
76	22.3	23.3	23.1	22.9	0.5	22.8	23.7	23.7	23.4	0.5
77	21.9	22.9	23.2	22.7	0.7	22.2	23.4	23.4	23.0	0.7
78	21.9	22.6	22.9	22.5	0.5	22.4	22.6	23.6	22.9	0.6
79	21.2	22.5	23.5	22.4	1.2	21.6	23.1	23.1	22.6	0.9
80	22.2	22.5	22.5	22.4	0.2	22.7	22.1	22.9	22.6	0.4
81										
82										
83	21.7	22.8	22.8	22.4	0.6	21.9	22.9	23.0	22.6	0.6
84	21.7	23.0	22.7	22.5	0.7	22.4	23.1	23.2	22.9	0.4
85	21.3	22.9	22.8	22.3	0.9	21.6	23.4	23.5	22.8	1.1
86	22.3	22.5	22.7	22.5	0.2	23.0	23.3	23.2	23.2	0.2
87	20.8	22.6		21.7	1.3	21.4	23.1		22.3	1.2
88										
89										
90	21.8		22.8	22.3	0.7	21.5		23.3	22.4	1.3
91	21.1		22.7	21.9	1.1	21.7		23.2	22.5	1.1
92		23.1	23.2	23.2	0.1	23.2	23.2	22.8	23.1	0.2
93	20.7	24.9	21.9	22.5	2.2	21.1	22.7	22.7	22.2	0.9
94	20.4	21.6	21.8	21.3	0.8	21.0	22.7	22.8	22.2	1.0
95										
96										
97	21.5	22.6	22.7	22.3	0.7	21.8	23.1	23.4	22.8	0.9
98	21.6	22.6	22.8	22.3	0.6	21.7	23.1	23.3	22.7	0.9
99	21.9	22.5	22.9	22.4	0.5	22.4	23.2	23.2	22.9	0.5
100	21.9	21.9	21.9	21.9	0.0	22.7	22.7	22.7	22.7	0.0
101	20.1	21.7	21.5	21.1	0.9	20.4	22.6		21.5	1.6
102										
103										
104	21.1	22.4	22.6	22.0	0.8	21.6	23.2	23.3	22.7	1.0
105	22.1	22.8	22.9	22.6	0.4	22.4	23.0	23.1	22.8	0.4
106	20.2	21.6	21.7	21.2	0.8	20.9	22.6	22.4	22.0	0.9
AVG	21.5	22.7	22.5	**22.1**	**0.7**	22.0	23.1	23.1	**22.6**	**0.7**
stdev	0.7	0.6	0.6	73		0.6	0.4	0.4	73	

Table 9. Chamber Temperatures in the Mid and High Concentration Chambers

Study	Mid (700 mg/m^3)			Daily		High (2000 mg/m^3)			Daily	
Day	AM	Mid	PM	Avg	St.dev	AM	Mid	PM	Avg	St.dev
1	22.1		22.3	22.2	0.1	21.6		22.1	21.9	0.4
2	22.7		23.3	23.0	0.4	22.1		22.7	22.4	0.4
3	22.2		23.3	22.8	0.8	21.6		22.7	22.2	0.8
4										
5										
6	21.9			21.9		21.6			21.6	
7	21.1		21.8	21.5	0.5	21.4		22.1	21.8	0.5
8	21.6		23.1	22.4	1.1	21.8		22.6	22.2	0.6
9	21.7		23.3	22.5	1.1	21.9		22.8	22.4	0.6
10	22.5		23.3	22.9	0.6	21.7		22.7	22.2	0.7
11										
12										
13	22.1		22.8	22.5	0.5	21.6		22.6	22.1	0.7
14	19.2		20.5	19.9	0.9	19.1		20.8	20.0	1.2
15	18.8		20.2	19.5	1.0	19.0		20.5	19.8	1.1
16			24.6	24.6				23.6	23.6	
17	24.3		24.5	24.4	0.1	23.0		23.3	23.2	0.2
18										
19										
20										
21	23.9		22.9	23.4	0.7	22.6		22.7	22.7	0.1
22	22.4		23.4	22.9	0.7	21.7		22.8	22.3	0.8
23	21.7		23.5	22.6	1.3	21.4		22.8	22.1	1.0
24	23.1		23.2	23.2	0.1	22.4		22.7	22.6	0.2
25										
26										
27	22.4		24.0	23.2	1.1	21.8		22.9	22.4	0.8
28	22.1		23.4	22.8	0.9	21.4		22.9	22.2	1.1
29			23.0	23.0				22.8	22.8	
30	22.1		23.5	22.8	1.0	21.7		22.9	22.3	0.8
31	22.2		23.3	22.8	0.8	21.6		22.7	22.2	0.8
32										
33										
34	22.4		23.6	23.0	0.8	21.8		22.7	22.3	0.6
35	22.6		23.6	23.1	0.7	21.8		22.8	22.3	0.7
36	23.0		23.3	23.2	0.2	22.3		22.5	22.4	0.1
37	22.8		23.4	23.1	0.4	22.0		22.7	22.4	0.5
38										
39										

Table 9. Chamber Temperatures in the Mid and High Concentration Chambers (continued)

Table 9. Chamber Temperatures in the Mid and High Concentration Chambers (continued).

Study	Mid (700 mg/m^3)			Daily		High (2000 mg/m^3)			Daily	
Day	AM	Mid	PM	Avg	St.dev	AM	Mid	PM	Avg	St.dev
41	22.2		23.3	22.8	0.8	21.5		22.7	22.1	0.8
42	22.6		23.0	22.8	0.3	22.5		22.8	22.7	0.2
43	22.9		23.4	23.2	0.4	22.0		22.7	22.4	0.5
44	21.6		23.0	22.3	1.0	21.3		22.7	22.0	1.0
45	22.8		23.4	23.1	0.4	22.2		22.8	22.5	0.4
46										
47										
48	22.4		23.5	23.0	0.8	21.7		22.9	22.3	0.8
49	22.1		23.4	22.8	0.9	21.2		23.1	22.2	1.3
50	22.0		23.6	22.8	1.1	21.6		23.1	22.4	1.1
51	21.7	23.0	23.4	22.7	0.9	21.3	22.6	22.9	22.3	0.9
52	22.4	23.3	22.8	22.8	0.5	21.7	22.7	22.6	22.3	0.6
53										
54										
55										
56	21.9	23.4	23.2	22.8	0.8	21.4	22.8	22.9	22.4	0.8
57	22.7	23.6	22.7	23.0	0.5	22.1	23.1	22.8	22.7	0.5
58	21.9	23.3	23.1	22.8	0.8	21.1	22.4	22.7	22.1	0.9
59	21.2	22.8	23.3	22.4	1.1	21.2	22.3	22.8	22.1	0.8
60										
61										
62	22.5	22.9	22.8	22.7	0.2	22.1	22.6	22.8	22.5	0.4
63	22.5	22.1	22.1	22.2	0.2	21.6	22.0	22.3	22.0	0.4
64	22.2	23.6	23.5	23.1	0.8	21.6	23.9	23.2	22.9	1.2
65	22.0	23.8	23.9	23.2	1.1	21.8	23.3	23.3	22.8	0.9
66	22.4	23.6	23.8	23.3	0.8	21.8	22.9	23.3	22.7	0.8
67										
68										
69	23.1	23.1	23.2	23.1	0.1	22.8	23.1	23.1	23.0	0.2
70	22.1	22.9	22.5	22.5	0.4	22.0	23.0	22.8	22.6	0.5
71	22.2	22.6	23.0	22.6	0.4	22.0	22.8	23.1	22.6	0.6
72	22.7	22.8	22.2	22.6	0.3	22.1	22.9	22.7	22.6	0.4
73	21.8	23.5	23.4	22.9	1.0	21.6	23.0	22.9	22.5	0.8
74										
75										

Table 9. Chamber Temperatures in the Mid and High Concentration Chambers (continued)

Study	Mid (700 mg/m^3)			Daily		High (2000 mg/m^3)			Daily	
Day	AM	Mid	PM	Avg	St.dev	AM	Mid	PM	Avg	St.dev
76	23.2	23.4	23.4	23.3	0.1	22.5	22.9	23.1	22.8	0.3
77	22.2	23.0	23.0	22.7	0.5	21.8	22.9	22.9	22.5	0.6
78	22.8	22.7	22.7	22.7	0.1	22.1	22.4	22.7	22.4	0.3
79	21.6	23.0	22.4	22.3	0.7	21.2	22.8	22.6	22.2	0.9
80	22.4	22.7	22.9	22.7	0.3	22.3	22.6	23.1	22.7	0.4
81										
82										
83	22.1	22.4	22.8	22.4	0.4	21.6	22.4	22.6	22.2	0.5
84	21.8	22.1	22.3	22.1	0.3	21.8	22.6	22.7	22.4	0.5
85	21.6	22.6	22.8	22.3	0.6	21.0	22.7	22.9	22.2	1.0
86	22.9	23.2	22.7	22.9	0.3	22.6	22.8	22.7	22.7	0.1
87	21.7	23.4		22.6	1.2	21.4	22.7		22.1	0.9
88										
89										
90	22.6		23.0	22.8	0.3	21.7		22.8	22.3	0.8
91	22.4		22.3	22.4	0.1	21.4		22.3	21.9	0.6
92	23.3		21.8	22.6	1.1		22.8	22.1	22.5	0.5
93	20.8	23.1	23.1	22.3	1.3	20.7	22.4	22.4	21.8	1.0
94	21.2	23.0	23.1	22.4	1.1	20.9	22.4	22.4	21.9	0.9
95										
96										
97	22.2	23.3	23.7	23.1	0.8	21.5	22.6	22.9	22.3	0.7
98	22.1	23.4	23.6	23.0	0.8	21.3	22.6	22.9	22.3	0.9
99	22.7	23.3	23.3	23.1	0.3	21.9	22.8	22.8	22.5	0.5
100	22.9	22.9	22.9	22.9	0.0	22.4	22.4	22.4	22.4	0.0
101	21.2	22.4	22.4	22.0	0.7	20.6	22.1	22.1	21.6	0.9
102										
103										
104	21.9	23.4	23.5	22.9	0.9	20.9	23.0	23.1	22.3	1.2
105	22.7	23.6	23.6	23.3	0.5	21.9	22.8	22.8	22.5	0.5
106	21.4	23.3	21.3	22.0	1.1	20.7	22.5	21.8	21.7	0.9
AVG	22.2	23.1	23.0	**22.7**	**0.6**	21.6	22.7	22.7	**22.3**	**0.7**
stdev	0.8	0.4	0.7	73		0.7	0.3	0.5	73	

APPENDIX C. IN-LIFE DATA

Table 1. Individual body Weights, Males

	Study Day	-7	1	8	15	22	29
Animal #	**Group**	**5/4/2011**	**5/11/2011**	**5/18/2011**	**5/25/2011**	**6/1/2011**	**6/8/2011**
1	C	192	211	226	241	247	262
3	C	175	202	222	234	249	262
5	C	155	179	200	221	231	252
7	C	166	186	206	218	231	242
9	C	190	221	238	250	266	276
11	C	161	188	209	219	232	248
13	C	202	235	267	290	304	320
15	C	201	232	250	265	278	293
17	C	181	212	231	248	258	268
19	C	159	180	200	217	232	246
21	L	193	220	231	252	263	279
23	L	185	212	228	247	260	273
25	L	174	200	221	240	250	256
27	L	171	195	216	229	237	247
29	L	191	219	234	251	262	273
31	L	189	213	234	250	256	261
33	L	184	214	228	246	257	266
35	L	183	209	234	250	260	269
37	L	197	223	245	260	277	292
39	L	183	209	233	251	261	276
41	M	158	182	200	217	231	250
43	M	174	200	218	229	244	256
45	M	177	201	223	232	248	259
47	M	172	194	213	222	239	252
49	M	161	182	197	211	225	243
51	M	188	210	236	249	264	277
53	M	180	205	236	250	268	281
55	M	173	195	218	233	242	257
57	M	172	195	230	247	251	249
59	M	171	194	216	232	237	260
61	H	165	195	215	227	242	257
63	H	177	197	211	220	238	249
65	H	172	197	216	226	234	243
67	H	186	212	230	247	256	271
69	H	160	187	204	219	232	242
71	H	171	197	217	230	235	243
73	H	148	173	188	201	211	218
75	H	178	211	229	239	254	264
77	H	177	197	214	221	229	241
79	H	178	209	229	242	255	271

Table 1. Individual body Weights, Males (continued)

Animal #	Study Day Group	36 **6/15/2011**	43 **6/22/2011**	50 **6/29/2011**	57 **7/6/2011**	64 **7/13/2011**	71 **7/20/2011**
1	C	270	284	286	294	301	307
3	C	271	274	281	292	297	310
5	C	274	285	291	306	309	320
7	C	247	261	268	275	284	291
9	C	286	296	300	309	311	313
11	C	265	275	285	292	296	307
13	C	340	354	360	367	380	393
15	C	307	315	321	334	344	345
17	C	284	288	298	308	319	322
19	C	258	274	286	296	306	315
21	L	290	298	305	317	326	305
23	L	285	288	290	300	311	302
25	L	265	270	280	292	298	293
27	L	260	266	277	290	303	293
29	L	285	288	299	313	318	307
31	L	274	278	291	302	312	299
33	L	282	295	300	312	322	310
35	L	282	289	295	310	317	303
37	L	302	311	315	324	332	324
39	L	288	303	318	328	336	329
41	M	259	297	285	300	306	316
43	M	272	275	283	292	298	305
45	M	274	281	285	294	301	308
47	M	259	267	279	293	301	307
49	M	256	262	279	292	301	312
51	M	288	277	304	318	325	332
53	M	289	291	302	313	323	330
55	M	267	278	288	301	307	317
57	M	264	264	275	290	301	309
59	M	266	275	278	290	299	303
61	H	273	287	296	308	314	318
63	H	259	270	274	283	290	297
65	H	253	264	275	287	295	301
67	H	285	291	301	314	320	324
69	H	259	266	278	289	294	299
71	H	256	264	275	284	291	296
73	H	225	236	243	263	268	279
75	H	277	285	298	311	318	322
77	H	250	261	264	280	283	291
79	H	286	291	303	316	324	335

Table 1. Individual body Weights, Males (continued)

Animal #	Study Day Group	78 **7/27/2011**	85 **8/3/2011**	92 **8/10/2011**	99 **8/17/2011**	106 **8/24/2011**	107 **8/25/2011**
1	C	316	318	321	330	318	
3	C	314	321	324	331	318	
5	C	329	331	328	330	316	
7	C	289	303	309	314	298	
9	C	322	329	329	331	316	
11	C	315	321	331	327	331	318
13	C	406	407	410	418	418	405
15	C	350	360	367	370	372	356
17	C	326	332	340	346	347	327
19	C	330	336	341	347	345	328
21	L	324	337	345	340	338	
23	L	316	322	330	335	324	
25	L	309	322	322	324	310	
27	L	309	325	331	333	315	
29	L	324	338	341	344	329	
31	L	316	327	335	339	339	326
33	L	329	337	350	349	350	336
35	L	322	331	334	337	335	322
37	L	333	343	349	355	360	337
39	L	345	361	363	368	371	350
41	M	318	325	325	332	319	
43	M	307	310	303	316	303	
45	M	310	316	318	324	309	
47	M	311	318	327	328	312	
49	M	323	324	334	337	320	
51	M	339	345	347	348	354	339
53	M	339	340	347	354	359	343
55	M	328	330	342	344	350	331
57	M	316	319	322	328	335	316
59	M	312	320	325	337	340	317
61	H	328	336	337	345		
63	H	300	309	310	312	298	
65	H	307	321	316	319	331	
67	H	328	339	337	347	304	
69	H	307	319	324	328	301	
71	H	305	303	307	303	308	291
73	H	287	291	297	310	308	292
75	H	329	331	337	339	339	317
77	H	297	302	314	312	317	297
79	H	341	350	356	363	365	346

Table 2. Individual Body Weights, Female

Animal #	Study Day Group	-7 **5/4/2011**	1 **5/11/2011**	8 **5/18/2011**	15 **5/25/2011**	22 **6/1/2011**	29 **6/8/2011**
2	C	118	132	140	148	158	161
4	C	125	138	152	159	165	173
6	C	132	151	160	169	172	180
66	C	104	119	127	136	136	139
10	C	112	123	135	144	148	155
12	C	115	128	137	143	148	153
14	C	125	140	150	152	154	162
16	C	114	127	134	143	148	152
18	C	110	127	135	140	145	153
20	C	119	134	138	143	154	158
22	L	131	150	159	170	169	177
24	L	107	134	136	144	151	154
26	L	116	125	144	153	163	166
28	L	120	137	145	151	158	164
30	L	118	137	149	155	163	170
32	L	122	135	149	154	166	171
34	L	123	140	149	155	160	164
36	L	121	139	149	155	162	163
38	L	102	116	123	133	140	146
40	L	121	135	147	154	160	164
42	M	126	144	151	160	164	171
44	M	115	132	142	152	158	161
46	M	105	121	129	135	140	147
48	M	110	130	142	150	156	158
50	M	116	132	143	154	162	170
52	M	123	135	148	151	161	167
54	M	116	128	135	142	149	150
56	M	129	142	152	154	157	160
58	M	126	140	154	159	166	169
60	M	109	123	132	139	141	145
62	H	111	126	132	135	143	148
64	H	113	127	136	142	148	154
8	H	113	127	136	144	148	152
68	H	123	138	143	146	154	158
70	H	116	131	142	148	151	155
72	H	117	129	137	141	147	152
74	H	116	135	145	154	157	163
76	H	113	128	138	147	149	151
78	H	113	132	135	143	149	152
80	H	116	129	139	148	152	155

Table 2. Individual Body Weights, Female (continued)

Animal #	Study Day / Group	36 **6/15/2011**	43 **6/22/2011**	50 **6/29/2011**	57 **7/6/2011**	64 **7/13/2011**	71 **7/20/2011**
2	C	164	171	174	175	179	181
4	C	177	179	182	185	191	191
6	C	181	191	197	197	199	202
66	C	140	152	153	160	161	161
10	C	159	164	166	169	174	175
12	C	157	160	165	167	165	171
14	C	166	164	171	171	179	180
16	C	160	159	165	169	171	169
18	C	159	160	167	168	167	173
20	C	158	164	164	169	169	172
22	L	178	185	184	189	193	180
24	L	160	160	165	173	178	173
26	L	174	181	183	183	184	180
28	L	170	166	174	179	178	174
30	L	175	178	184	187	190	184
32	L	176	181	185	190	192	183
34	L	169	170	172	180	182	173
36	L	166	170	174	182	186	184
38	L	145	150	156	163	166	162
40	L	180	178	179	186	189	185
42	M	180	174	183	190	189	192
44	M	165	165	173	181	181	186
46	M	151	155	159	163	168	168
48	M	158	163	164	169	172	174
50	M	178	182	182	188	190	186
52	M	173	181	175	184	185	185
54	M	161	165	166	175	175	178
56	M	162	170	170	173	177	174
58	M	175	181	184	189	189	189
60	M	149	153	159	161	164	165
62	H	152	154	156	164	164	162
64	H	162	164	167	171	173	178
8	H	155	160	166	167	170	179
68	H	160	165	165	171	172	172
70	H	158	160	167	173	172	175
72	H	151	156	164	169	169	174
74	H	167	170	175	180	184	184
76	H	158	162	168	174	175	174
78	H	154	159	161	168	171	174
80	H	157	164	172	187	177	175

Table 2. Individual Body Weights, Female (continued)

Animal #	Study Day / Group	78 **7/27/2011**	85 **8/3/2011**	92 **8/10/2011**	99 **8/17/2011**	106 **8/24/2011**	107 **8/25/2011**
2	C	183	183	184	187	179	
4	C	190	192	194	192	183	
6	C	208	209	215	213	202	
66	C	166	167	173	174	164	
10	C	176	181	182	181	169	
12	C	176	175	181	181	180	174
14	C	177	183	183	191	191	184
16	C	176	176	180	182	180	171
18	C	172	171	175	174	184	170
20	C	174	175	179	178	176	166
22	L	191	194	193	194	187	
24	L	176	185	185	187	179	
26	L	188	190	191	197	186	
28	L	183	189	185	187	177	
30	L	189	196	194	199	190	
32	L	189	194	202	200	199	188
34	L	179	185	187	187	186	176
36	L	185	187	189	194	192	181
38	L	169	170	174	175	178	168
40	L	185	198	198	208	200	191
42	M	193	196	198	200	190	
44	M	190	192	190	193	183	
46	M	175	171	178	177		
48	M	179	186	187	187	177	
50	M	191	194	197	200	186	
52	M	189	190	193	194	200	186
54	M	179	181	184	184	187	176
56	M	181	184	186	187	187	175
58	M	190	197	200	202	202	191
60	M	167	170	172	170	177	166
62	H	167	164	167	166	160	
64	H	180	179	181	187	176	
8	H	173	177	176	176	162	
68	H	177	177	179	182	170	
70	H	174	177	182	182	168	
72	H	175	170	180	175	177	167
74	H	187	188	189	191	196	188
76	H	175	174	181	183	182	179
78	H	173	173	177	179	178	166
80	H	175	179	180	185	184	172

Table 3. Individual Body Weight Gain, Males

	Study Day	1	8	15	22	29
Animal #	Group	5/11/2011	5/18/2011	5/25/2011	6/1/2011	6/8/2011
1	C	2.7	2.1	2.1	0.9	2.1
3	C	3.9	2.9	1.7	2.1	1.9
5	C	3.4	3.0	3.0	1.4	3.0
7	C	2.9	2.9	1.7	1.9	1.6
9	C	4.4	2.4	1.7	2.3	1.4
11	C	3.9	3.0	1.4	1.9	2.3
13	C	4.7	4.6	3.3	2.0	2.3
15	C	4.4	2.6	2.1	1.9	2.1
17	C	4.4	2.7	2.4	1.4	1.4
19	C	3.0	2.9	2.4	2.1	2.0
21	L	3.9	1.6	3.0	1.6	2.3
23	L	3.9	2.3	2.7	1.9	1.9
25	L	3.7	3.0	2.7	1.4	0.9
27	L	3.4	3.0	1.9	1.1	1.4
29	L	4.0	2.1	2.4	1.6	1.6
31	L	3.4	3.0	2.3	0.9	0.7
33	L	4.3	2.0	2.6	1.6	1.3
35	L	3.7	3.6	2.3	1.4	1.3
37	L	3.7	3.1	2.1	2.4	2.1
39	L	3.7	3.4	2.6	1.4	2.1
41	M	3.4	2.6	2.4	2.0	2.7
43	M	3.7	2.6	1.6	2.1	1.7
45	M	3.4	3.1	1.3	2.3	1.6
47	M	3.1	2.7	1.3	2.4	1.9
49	M	3.0	2.1	2.0	2.0	2.6
51	M	3.1	3.7	1.9	2.1	1.9
53	M	3.6	4.4	2.0	2.6	1.9
55	M	3.1	3.3	2.1	1.3	2.1
57	M	3.3	5.0	2.4	0.6	-0.3
59	M	3.3	3.1	2.3	0.7	3.3
61	H	4.3	2.9	1.7	2.1	2.1
63	H	2.9	2.0	1.3	2.6	1.6
65	H	3.6	2.7	1.4	1.1	1.3
67	H	3.7	2.6	2.4	1.3	2.1
69	H	3.9	2.4	2.1	1.9	1.4
71	H	3.7	2.9	1.9	0.7	1.1
73	H	3.6	2.1	1.9	1.4	1.0
75	H	4.7	2.6	1.4	2.1	1.4
77	H	2.9	2.4	1.0	1.1	1.7
79	H	4.4	2.9	1.9	1.9	2.3

Table 3. Individual Body Weight Gain, Males (continued)

	Study Day	36	43	50	57	64	71
Animal #	Group	6/15/2011	6/22/2011	6/29/2011	7/6/2011	7/13/2011	7/20/2011
1	C	1.1	2.0	0.3	1.1	1.0	0.9
3	C	1.3	0.4	1.0	1.6	0.7	1.9
5	C	3.1	1.6	0.9	2.1	0.4	1.6
7	C	0.7	2.0	1.0	1.0	1.3	1.0
9	C	1.4	1.4	0.6	1.3	0.3	0.3
11	C	2.4	1.4	1.4	1.0	0.6	1.6
13	C	2.9	2.0	0.9	1.0	1.9	1.9
15	C	2.0	1.1	0.9	1.9	1.4	0.1
17	C	2.3	0.6	1.4	1.4	1.6	0.4
19	C	1.7	2.3	1.7	1.4	1.4	1.3
21	L	1.6	1.1	1.0	1.7	1.3	-3.0
23	L	1.7	0.4	0.3	1.4	1.6	-1.3
25	L	1.3	0.7	1.4	1.7	0.9	-0.7
27	L	1.9	0.9	1.6	1.9	1.9	-1.4
29	L	1.7	0.4	1.6	2.0	0.7	-1.6
31	L	1.9	0.6	1.9	1.6	1.4	-1.9
33	L	2.3	1.9	0.7	1.7	1.4	-1.7
35	L	1.9	1.0	0.9	2.1	1.0	-2.0
37	L	1.4	1.3	0.6	1.3	1.1	-1.1
39	L	1.7	2.1	2.1	1.4	1.1	-1.0
41	M	1.3	5.4	-1.7	2.1	0.9	1.4
43	M	2.3	0.4	1.1	1.3	0.9	1.0
45	M	2.1	1.0	0.6	1.3	1.0	1.0
47	M	1.0	1.1	1.7	2.0	1.1	0.9
49	M	1.9	0.9	2.4	1.9	1.3	1.6
51	M	1.6	-1.6	3.9	2.0	1.0	1.0
53	M	1.1	0.3	1.6	1.6	1.4	1.0
55	M	1.4	1.6	1.4	1.9	0.9	1.4
57	M	2.1	0.0	1.6	2.1	1.6	1.1
59	M	0.9	1.3	0.4	1.7	1.3	0.6
61	H	2.3	2.0	1.3	1.7	0.9	0.6
63	H	1.4	1.6	0.6	1.3	1.0	1.0
65	H	1.4	1.6	1.6	1.7	1.1	0.9
67	H	2.0	0.9	1.4	1.9	0.9	0.6
69	H	2.4	1.0	1.7	1.6	0.7	0.7
71	H	1.9	1.1	1.6	1.3	1.0	0.7
73	H	1.0	1.6	1.0	2.9	0.7	1.6
75	H	1.9	1.1	1.9	1.9	1.0	0.6
77	H	1.3	1.6	0.4	2.3	0.4	1.1
79	H	2.1	0.7	1.7	1.9	1.1	1.6

Table 3. Individual Body Weight Gain, Males (continued)

Animal #	Study Day / Group	78 7/27/2011	85 8/3/2011	92 8/10/2011	99 8/17/2011	106 8/24/2011	107 8/25/2011
1	C	1.3	0.3	0.4	1.3	-1.7	
3	C	0.6	1.0	0.4	1.0	-1.9	
5	C	1.3	0.3	-0.4	0.3	-2.0	
7	C	-0.3	2.0	0.9	0.7	-2.3	
9	C	1.3	1.0	0.0	0.3	-2.1	
11	C	1.1	0.9	1.4	-0.6	0.6	-1.9
13	C	1.9	0.1	0.4	1.1	0.0	-1.9
15	C	0.7	1.4	1.0	0.4	0.3	-2.3
17	C	0.6	0.9	1.1	0.9	0.1	-2.9
19	C	2.1	0.9	0.7	0.9	-0.3	-2.4
21	L	2.7	1.9	1.1	-0.7	-0.3	
23	L	2.0	0.9	1.1	0.7	-1.6	
25	L	2.3	1.9	0.0	0.3	-2.0	
27	L	2.3	2.3	0.9	0.3	-2.6	
29	L	2.4	2.0	0.4	0.4	-2.1	
31	L	2.4	1.6	1.1	0.6	0.0	-1.9
33	L	2.7	1.1	1.9	-0.1	0.1	-2.0
35	L	2.7	1.3	0.4	0.4	-0.3	-1.9
37	L	1.3	1.4	0.9	0.9	0.7	-3.3
39	L	2.3	2.3	0.3	0.7	0.4	-3.0
41	M	0.3	1.0	0.0	1.0	-1.9	
43	M	0.3	0.4	-1.0	1.9	-1.9	
45	M	0.3	0.9	0.3	0.9	-2.1	
47	M	0.6	1.0	1.3	0.1	-2.3	
49	M	1.6	0.1	1.4	0.4	-2.4	
51	M	1.0	0.9	0.3	0.1	0.9	-2.1
53	M	1.3	0.1	1.0	1.0	0.7	-2.3
55	M	1.6	0.3	1.7	0.3	0.9	-2.7
57	M	1.0	0.4	0.4	0.9	1.0	-2.7
59	M	1.3	1.1	0.7	1.7	0.4	-3.3
61	H	1.4	1.1	0.1	1.1		
63	H	0.4	1.3	0.1	0.3	-2.0	
65	H	0.9	2.0	-0.7	0.4	1.7	
67	H	0.6	1.6	-0.3	1.4	-6.1	
69	H	1.1	1.7	0.7	0.6	-3.9	
71	H	1.3	-0.3	0.6	-0.6	0.7	-2.4
73	H	1.1	0.6	0.9	1.9	-0.3	-2.3
75	H	1.0	0.3	0.9	0.3	0.0	-3.1
77	H	0.9	0.7	1.7	-0.3	0.7	-2.9
79	H	0.9	1.3	0.9	1.0	0.3	-2.7

Table 4. Individual Body Weight Gain, Females

	Study Day	1	8	15	22	29
Animal #	Group	5/11/2011	5/18/2011	5/25/2011	6/1/2011	6/8/2011
2	C	2.0	1.1	1.1	1.4	0.4
4	C	1.9	2.0	1.0	0.9	1.1
6	C	2.7	1.3	1.3	0.4	1.1
66	C	2.1	1.1	1.3	0.0	0.4
10	C	1.6	1.7	1.3	0.6	1.0
12	C	1.9	1.3	0.9	0.7	0.7
14	C	2.1	1.4	0.3	0.3	1.1
16	C	1.9	1.0	1.3	0.7	0.6
18	C	2.4	1.1	0.7	0.7	1.1
20	C	2.1	0.6	0.7	1.6	0.6
22	L	2.7	1.3	1.6	-0.1	1.1
24	L	3.9	0.3	1.1	1.0	0.4
26	L	1.3	2.7	1.3	1.4	0.4
28	L	2.4	1.1	0.9	1.0	0.9
30	L	2.7	1.7	0.9	1.1	1.0
32	L	1.9	2.0	0.7	1.7	0.7
34	L	2.4	1.3	0.9	0.7	0.6
36	L	2.6	1.4	0.9	1.0	0.1
38	L	2.0	1.0	1.4	1.0	0.9
40	L	2.0	1.7	1.0	0.9	0.6
42	M	2.6	1.0	1.3	0.6	1.0
44	M	2.4	1.4	1.4	0.9	0.4
46	M	2.3	1.1	0.9	0.7	1.0
48	M	2.9	1.7	1.1	0.9	0.3
50	M	2.3	1.6	1.6	1.1	1.1
52	M	1.7	1.9	0.4	1.4	0.9
54	M	1.7	1.0	1.0	1.0	0.1
56	M	1.9	1.4	0.3	0.4	0.4
58	M	2.0	2.0	0.7	1.0	0.4
60	M	2.0	1.3	1.0	0.3	0.6
62	H	2.1	0.9	0.4	1.1	0.7
64	H	2.0	1.3	0.9	0.9	0.9
8	H	2.0	1.3	1.1	0.6	0.6
68	H	2.1	0.7	0.4	1.1	0.6
70	H	2.1	1.6	0.9	0.4	0.6
72	H	1.7	1.1	0.6	0.9	0.7
74	H	2.7	1.4	1.3	0.4	0.9
76	H	2.1	1.4	1.3	0.3	0.3
78	H	2.7	0.4	1.1	0.9	0.4
80	H	1.9	1.4	1.3	0.6	0.4

Table 4. Individual Body Weight Gain, Females (continued)

	Study Day	36	43	50	57	64	71
Animal #	Group	6/15/2011	6/22/2011	6/29/2011	7/6/2011	7/13/2011	7/20/2011
2	C	0.4	1.0	0.4	0.1	0.6	0.3
4	C	0.6	0.3	0.4	0.4	0.9	0.0
6	C	0.1	1.4	0.9	0.0	0.3	0.4
66	C	0.1	1.7	0.1	1.0	0.1	0.0
10	C	0.6	0.7	0.3	0.4	0.7	0.1
12	C	0.6	0.4	0.7	0.3	-0.3	0.9
14	C	0.6	-0.3	1.0	0.0	1.1	0.1
16	C	1.1	-0.1	0.9	0.6	0.3	-0.3
18	C	0.9	0.1	1.0	0.1	-0.1	0.9
20	C	0.0	0.9	0.0	0.7	0.0	0.4
22	L	0.1	1.0	-0.1	0.7	0.6	-1.9
24	L	0.9	0.0	0.7	1.1	0.7	-0.7
26	L	1.1	1.0	0.3	0.0	0.1	-0.6
28	L	0.9	-0.6	1.1	0.7	-0.1	-0.6
30	L	0.7	0.4	0.9	0.4	0.4	-0.9
32	L	0.7	0.7	0.6	0.7	0.3	-1.3
34	L	0.7	0.1	0.3	1.1	0.3	-1.3
36	L	0.4	0.6	0.6	1.1	0.6	-0.3
38	L	-0.1	0.7	0.9	1.0	0.4	-0.6
40	L	2.3	-0.3	0.1	1.0	0.4	-0.6
42	M	1.3	-0.9	1.3	1.0	-0.1	0.4
44	M	0.6	0.0	1.1	1.1	0.0	0.7
46	M	0.6	0.6	0.6	0.6	0.7	0.0
48	M	0.0	0.7	0.1	0.7	0.4	0.3
50	M	1.1	0.6	0.0	0.9	0.3	-0.6
52	M	0.9	1.1	-0.9	1.3	0.1	0.0
54	M	1.6	0.6	0.1	1.3	0.0	0.4
56	M	0.3	1.1	0.0	0.4	0.6	-0.4
58	M	0.9	0.9	0.4	0.7	0.0	0.0
60	M	0.6	0.6	0.9	0.3	0.4	0.1
62	H	0.6	0.3	0.3	1.1	0.0	-0.3
64	H	1.1	0.3	0.4	0.6	0.3	0.7
8	H	0.4	0.7	0.9	0.1	0.4	1.3
68	H	0.3	0.7	0.0	0.9	0.1	0.0
70	H	0.4	0.3	1.0	0.9	-0.1	0.4
72	H	-0.1	0.7	1.1	0.7	0.0	0.7
74	H	0.6	0.4	0.7	0.7	0.6	0.0
76	H	1.0	0.6	0.9	0.9	0.1	-0.1
78	H	0.3	0.7	0.3	1.0	0.4	0.4
80	H	0.3	1.0	1.1	2.1	-1.4	-0.3

Table 4. Individual Body Weight Gain, Females (continued)

	Study Day	78	85	92	99	106	107
Animal #	Group	7/27/2011	8/3/2011	8/10/2011	8/17/2011	8/24/2011	8/25/2011
2	C	0.3	0.0	0.1	0.4	-1.1	
4	C	-0.1	0.3	0.3	-0.3	-1.3	
6	C	0.9	0.1	0.9	-0.3	-1.6	
66	C	0.7	0.1	0.9	0.1	-1.4	
10	C	0.1	0.7	0.1	-0.1	-1.7	
12	C	0.7	-0.1	0.9	0.0	-0.1	-0.9
14	C	-0.4	0.9	0.0	1.1	0.0	-1.0
16	C	1.0	0.0	0.6	0.3	-0.3	-1.3
18	C	-0.1	-0.1	0.6	-0.1	1.4	-2.0
20	C	0.3	0.1	0.6	-0.1	-0.3	-1.4
22	L	1.6	0.4	-0.1	0.1	-1.0	
24	L	0.4	1.3	0.0	0.3	-1.1	
26	L	1.1	0.3	0.1	0.9	-1.6	
28	L	1.3	0.9	-0.6	0.3	-1.4	
30	L	0.7	1.0	-0.3	0.7	-1.3	
32	L	0.9	0.7	1.1	-0.3	-0.1	-1.6
34	L	0.9	0.9	0.3	0.0	-0.1	-1.4
36	L	0.1	0.3	0.3	0.7	-0.3	-1.6
38	L	1.0	0.1	0.6	0.1	0.4	-1.4
40	L	0.0	1.9	0.0	1.4	-1.1	-1.3
42	M	0.1	0.4	0.3	0.3	-1.4	
44	M	0.6	0.3	-0.3	0.4	-1.4	
46	M	1.0	-0.6	1.0	-0.1		
48	M	0.7	1.0	0.1	0.0	-1.4	
50	M	0.7	0.4	0.4	0.4	-2.0	
52	M	0.6	0.1	0.4	0.1	0.9	-2.0
54	M	0.1	0.3	0.4	0.0	0.4	-1.6
56	M	1.0	0.4	0.3	0.1	0.0	-1.7
58	M	0.1	1.0	0.4	0.3	0.0	-1.6
60	M	0.3	0.4	0.3	-0.3	1.0	-1.6
62	H	0.7	-0.4	0.4	-0.1		
64	H	0.3	-0.1	0.3	0.9	-1.6	
8	H	-0.9	0.6	-0.1	0.0	-2.0	
68	H	0.7	0.0	0.3	0.4	-1.7	
70	H	-0.1	0.4	0.7	0.0	-2.0	
72	H	0.1	-0.7	1.4	-0.7	0.3	-1.4
74	H	0.4	0.1	0.1	0.3	0.7	-1.1
76	H	0.1	-0.1	1.0	0.3	-0.1	-0.4
78	H	-0.1	0.0	0.6	0.3	-0.1	-1.7
80	H	0.0	0.6	0.1	0.7	-0.1	-1.7

Table 5. Daily Food Consumption, Males

Animal No	Exp Group	5/11-13 Wk 1	5/16-20 Wk 2	5/23-27 Wk 3	5/30-6/3 Wk 4	6/6-10 Wk 5	6/13-17 Wk 6	6/20-24 Wk 7	6/27-7/1 Wk 8
1	C	12.4	16.3	13.9	16.4	15.8	16.0	16.0	15.7
3	C	13.0	16.6	14.2	16.6	15.9	15.8	14.4	15.7
5	C	9.5	14.8	14.0	16.3	16.2	17.9	17.3	17.5
7	C	12.3	15.1	12.3	14.5	14.1	14.7	14.2	15.1
9	C	13.8	18.5	16.4	18.6	16.9	17.2	16.7	16.7
11	C	11.3	15.7	12.9	15.6	15.9	17.1	16.0	16.3
13	C	16.9	20.9	18.7	19.6	19.8	19.9	20.1	20.0
15	C	14.4	18.8	16.0	18.4	19.0	19.5	16.9	18.1
17	C	12.6	18.1	15.5	17.6	16.6	17.4	16.7	17.0
19	C	9.4	14.2	13.6	15.6	14.8	17.0	16.8	17.3
21	L	15.0	18.2	15.3	18.2	16.2	17.2	12.8	18.1
23	L	10.3	16.7	14.9	17.6	16.2	16.2	15.8	16.7
25	L	13.5	17.3	14.8	17.3	14.3	14.3	14.6	16.3
27	L	13.0	16.7	15.0	16.0	13.1	14.4	13.7	16.4
29	L	12.6	16.6	15.0	17.8	15.5	16.6	15.0	17.1
31	L	14.1	17.8	15.7	17.5	15.0	16.0	15.2	17.5
33	L	10.7	17.0	14.1	17.7	16.7	16.6	15.8	17.8
35	L	13.3	17.3	14.6	17.9	15.0	15.5	15.1	17.4
37	L	16.1	19.8	17.0	20.2	17.4	18.3	17.8	19.7
39	L		17.5	15.6	18.7	16.2	16.9	17.5	19.5
41	M	12.0	15.2	12.6	15.9	15.3	15.1	15.7	18.2
43	M	12.1	16.8	14.7	17.4	16.5	16.3	15.8	16.9
45	M	11.5	16.2	14.3	17.0	15.5	16.1	15.8	16.4
47	M	9.2	14.8	12.8	16.1	14.4	14.5	14.6	17.3
49	M	9.9	13.6	12.6	15.2	15.0	15.7	14.6	17.9
51	M	11.8	18.0	15.6	18.3	16.7	16.8	16.1	17.2
53	M	13.2	19.1	17.2	19.9	17.5	16.9	17.2	17.2
55	M	11.1	16.0	14.1	16.7	14.8	15.2	15.0	16.8
57	M	12.7	18.2	15.1	17.1	12.2	14.4	13.8	14.7
59	M		17.2	14.2	16.4	15.9	15.5	14.7	17.4
61	H	10.1	15.0	13.3	15.9	15.3	16.1	16.1	18.3
63	H	8.6	16.3	14.8	17.5	15.8	15.4	15.8	16.4
65	H	10.3	16.6	14.7	17.1	13.5	14.2	14.6	16.5
67	H	11.7	17.2	14.3	16.3	15.1	16.4	17.2	16.9
69	H	9.9	14.9	13.2	15.8	14.7	16.1	15.4	17.0
71	H	10.3	15.6	13.7	15.7	14.7	15.4	14.4	17.4
73	H	9.1	12.9	11.5	13.2	11.9	12.3	12.1	16.1
75	H	11.4	16.8	14.6	16.9	15.4	16.0	16.1	17.2
77	H	12.4	15.6	12.6	13.6	12.9	15.8	14.2	16.1
79	H	6.7	15.4	15.9	18.2	17.2	17.8	16.9	17.9

Table 5. Daily Food Consumption, Males (continued)

Animal No	Exp Group	7/4-8 Wk 9	7/11-15 Wk 10	7/18-22 Wk 11	7/25-29 Wk 12	8/1-5 Wk 13	8/8-12 Wk 14	8/15-19 Wk 15	8/22-26 Wk 16
1	C	15.4	15.1	15.6	16.1	16.3	16.6	17.6	17.3
3	C	15.8	15.9	15.9	16.0	16.6	17.5	16.4	16.3
5	C	17.0	17.0	16.7	16.8	16.9	17.0	16.8	17.5
7	C	14.0	15.0	15.6	14.5	15.7	16.0	16.8	19.4
9	C	16.5	15.4	15.9	15.1	16.5	19.0	16.5	17.9
11	C	16.2	16.0	16.4	15.9	16.8	17.3	17.8	14.6
13	C	19.5	20.4	21.1	19.8	20.7	19.8	23.3	18.6
15	C	18.2	17.4	16.4	17.3	18.2	18.7	21.1	16.4
17	C	16.4	16.7	16.8	16.3	17.3	17.8	20.9	16.0
19	C	16.8	17.1	17.1	17.8	18.4	18.2	20.7	17.5
21	L	17.3	17.2	12.7	19.4	19.0	19.0	18.2	17.9
23	L	17.6	16.3	14.0	17.7	18.7	17.8	19.9	18.3
25	L	15.5	15.4	13.0	17.2	17.7	16.7	16.9	16.1
27	L	15.8	16.0	13.6	18.1	17.4	18.3	17.1	16.1
29	L	16.4	16.5	13.1	19.2	18.7	18.4	17.6	17.0
31	L	17.5	17.4	13.6	17.9	19.0	18.9	21.1	18.0
33	L	16.6	17.0	13.5	18.6	19.0	19.8	20.9	17.8
35	L	16.4	16.3	13.4	18.4	17.9	17.3	19.9	16.1
37	L	19.2	20.2	15.7	18.7	20.2	19.6	22.6	21.6
39	L	18.5	18.2	15.3	19.3	20.4	20.4	23.3	19.6
41	M	17.3	16.8	16.6	c	17.2	18.1	17.4	17.5
43	M	17.5	16.5	16.1	16.6	17.8	15.7	18.3	17.4
45	M	17.1	17.0	16.9	17.1	17.8	18.5	17.2	18.3
47	M	16.6	16.1	15.7	16.3	15.9	20.7	17.1	16.5
49	M	16.6	18.2	16.5	17.5	17.0	19.3	17.8	17.5
51	M	18.0	18.3	17.3	18.1	18.9	19.3	21.2	20.4
53	M	18.4	18.1	17.7	17.2	20.0	18.6	21.8	19.8
55	M	16.4	17.1	16.8	16.9	18.3	19.3	22.1	19.0
57	M	15.8	17.5	16.6	15.7	16.8	16.7	20.0	18.7
59	M	18.2	15.9	16.5	16.8	17.8	17.7	23.3	18.0
61	H	17.3	16.9	16.8	17.7	18.3	19.4	18.6	
63	H	16.2	16.4	16.6	16.7	17.1	19.2	17.0	5.3
65	H	16.1	16.3	16.1	17.4	18.0	18.2	18.8	11.2
67	H	17.8	17.7	18.0	17.0	18.6	18.8	19.5	16.0
69	H	16.8	16.3	15.6	17.7	19.1	18.7	17.7	15.6
71	H	15.6	15.7	16.1	15.9	16.7	16.2	18.5	16.6
73	H	14.2	18.4	14.6	15.7	15.9	16.8	19.5	15.9
75	H	17.0	16.4	15.6	17.2	17.0	17.1	20.5	17.2
77	H	16.9	15.7	14.8	15.3	16.8	17.1	18.6	16.6
79	H	19.1	18.5	18.5	19.5	19.8	20.1	22.1	19.3

Table 6. Daily Food Consumption, Females

Animal No	Exp Group	5/11-13 Wk 1	5/16-20 Wk 2	5/23-27 Wk 3	5/30-6/3 Wk 4	6/6-10 Wk 5	6/13-17 Wk 6	6/20-24 Wk 7	6/27-7/1 Wk 8
2	C	7.3	11.4	10.3	12.6	11.9	12.0	11.2	10.7
4	C	9.1	13.1	11.5	13.8	12.5	13.0	11.8	11.7
6	C	8.8	13.1	11.4	14.5	12.8	13.0	12.8	13.8
66	C	5.0	10.7	8.1	11.2	9.5	10.0	10.0	10.4
10	C	6.0	12.3	10.2	11.5	10.1	11.6	10.8	11.4
12	C	10.3	11.0	9.3	11.7	11.2	11.9	10.4	10.1
14	C	5.1	11.9	10.6	12.0	10.4	11.9	9.8	10.6
16	C	6.1	11.3	9.7	12.1	10.8	11.8	11.3	11.8
18	C	8.2	11.8	9.3	11.5	11.6	11.9	10.6	11.7
20	C	7.8	11.4	9.6	12.4	12.8	14.0	10.4	12.9
22	L	9.9	13.4	10.2	13.0	11.9	11.8	11.1	12.6
24	L	8.6	11.8	9.9	12.6	10.8	10.9	8.9	11.5
26	L	9.2	26.4	10.8	14.0	12.0	12.6	11.2	12.6
28	L	7.7	11.3	10.0	12.6	10.5	10.9	9.7	12.1
30	L	9.3	16.8	11.8	14.4	11.8	11.4	10.4	13.2
32	L	7.8	12.3	10.8	15.0	11.8	12.1	11.0	13.0
34	L	5.8	12.2	9.8	11.9	9.9	10.7	9.3	11.6
36	L	9.1	12.2	10.0	13.0	10.7	11.0	9.9	12.3
38	L	7.8	10.5	8.7	12.6	9.4	8.8	8.7	11.4
40	L	9.6	11.4	10.7	14.1	13.0	14.3	11.1	12.0
42	M	4.8	12.0	10.7	12.9	12.1	12.0	12.0	12.5
44	M	8.0	11.8	9.6	12.3	11.2	11.1	9.3	12.2
46	M	5.4	10.3	8.6	11.4	10.0	10.1	10.2	12.5
48	M	7.8	11.6	12.7	13.0	11.1	10.9	10.2	11.1
50	M	5.8	12.0	11.3	13.5	12.2	12.5	11.2	12.3
52	M	7.5	11.8	10.5	13.2	12.0	12.2	10.5	12.2
54	M	6.5	9.6	9.3	10.7	10.2	10.7	9.5	11.9
56	M	7.1	12.5	9.6	12.6	9.5	10.1	10.1	12.4
58	M	6.8	13.2	11.4	13.4	12.7	12.9	11.5	12.8
60	m	10.5	11.4	10.3	12.6	11.2	11.4	10.7	11.8
62	H	4.8	10.1	8.3	11.0	9.4	10.8	9.1	11.8
64	H	6.5	10.7	9.7	12.7	10.2	10.4	10.7	11.6
8	H	7.0	11.1	9.7	11.6	10.3	10.8	10.0	11.7
68	H	5.9	9.5	9.2	11.4	9.8	10.6	9.8	11.9
70	H	4.6	11.3	9.5	11.4	10.7	11.0	10.3	13.3
72	H	5.3	10.5	9.3	11.8	10.6	10.0	9.3	11.5
74	H	8.2	11.8	11.3	12.8	10.8	11.4	11.0	11.8
76	H	6.6	11.3	10.2	11.7	10.6	11.0	11.4	12.6
78	H	6.6	10.5	8.8	11.7	11.2	11.4	10.5	12.6
80	H	13.8	14.8	10.0	13.7	10.7	12.8	11.6	12.4

Table 6 . Daily Food Consumption, Females (continued)

Animal No	Exp Group	7/11-15 Wk 10	7/18-22 Wk 11	7/25-29 Wk 12	8/1-5 Wk 13	8/8-12 Wk 14	8/15-19 Wk 15	8/22-26 Wk 16
2	C	10.6	10.8	9.7	11.2	11.3	10.6	11.1
4	C	11.5	11.4	10.6	11.5	12.0	10.7	11.1
6	C	12.0	12.3	13.3	13.1	13.2	12.5	12.5
66	C	10.6	10.3	10.0	10.5	11.3	9.6	11.4
10	C	10.5	11.0	9.5	11.0	12.8	9.7	10.6
12	C	9.9	10.8	10.7	10.7	11.1	14.1	10.7
14	C	11.0	10.6	10.1	11.4	10.8	15.2	16.8
16	C	11.6	10.9	11.2	11.4	12.2	13.7	9.9
18	C	11.5	10.6	10.3	10.8	11.2	12.7	11.4
20	C	11.5	12.0	11.1	12.4	16.5	13.2	10.1
22	L	12.1	9.1	12.9	13.1	12.5	12.6	12.9
24	L	11.6	10.2	12.4	12.6	12.1	11.8	11.5
26	L	11.5	8.8	13.3	13.2	13.7	14.1	12.2
28	L	10.9	9.4	11.7	12.0	13.2	12.7	10.9
30	L	12.0	9.0	14.0	13.5	12.6	12.5	12.2
32	L	11.6	8.3	12.6	13.9	13.6	13.6	11.1
34	L	10.5	8.1	11.5	12.0	11.6	12.7	11.6
36	L	11.6	11.8	11.3	11.9	12.3	13.7	11.8
38	L	11.5	8.8	12.1	11.3	11.5	13.3	11.8
40	L	10.4	8.9	8.2	9.4	5.6		9.9
42	M	11.6	11.6	12.1	12.2	12.9	12.5	12.2
44	M	11.2	11.9	12.3	13.4	13.2	12.4	10.9
46	M	11.5	11.1	10.5	11.7	12.3	11.4	
48	M	11.4	11.6	11.6	12.4	13.0	11.8	11.4
50	M	12.6	11.8	11.5	13.1	14.1	13.1	11.5
52	M	12.4	11.8	13.1	12.7	13.5	13.7	13.9
54	M	11.3	10.5	11.8	11.3	12.5	13.4	12.4
56	M	12.1	10.9	12.8	12.4	13.7	13.9	11.4
58	M	13.1	12.8	12.7	13.7	14.0	15.4	14.7
60	m	12.1	11.7	11.5	11.4	12.9	14.5	13.5
62	H	10.6	10.3	10.3	10.5	11.5	11.0	25.6
64	H	11.2	11.4	12.1	10.9	15.8	14.3	17.2
8	H	12.3	11.9	11.5	12.4	12.2	13.0	7.1
68	H	10.6	10.6	11.9	11.5	20.9	11.4	29.2
70	H	11.2	11.4	12.0	13.0	13.5	12.1	14.7
72	H	11.6	11.0	10.9	10.3	11.6	12.7	11.4
74	H	11.8	11.6	13.3	13.2	12.7	14.5	13.3
76	H	12.5	12.3	11.4	13.2	12.4	14.8	12.0
78	H	11.8	11.3	11.4	11.6	12.6	14.0	12.6
80	H	8.8	8.8	11.4	12.3	11.8	13.6	13.0

Table 7. Organ Weights, Male (all weights in grams)

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
001	318	0.667	0.884	0.213	1.786	1.011	1.042
003	318	0.668	0.873	0.177	1.993	1.038	1.022
005	316	0.719	0.844	0.195	1.787	0.974	1.048
007	298	0.616	0.772	0.226	1.812	0.950	0.958
009	316	0.593	0.945	0.219	1.957	1.007	1.020
011	318	0.683	0.815	0.175	1.928	0.944	0.941
013	405	0.765	1.059	0.252	1.997	1.143	1.115
015	356	0.700	0.935	0.190	1.913	1.060	1.041
017	327	0.638	0.886	0.208	1.895	1.058	1.046
019	328	0.626	0.911	0.183	1.925	0.990	1.012
021	338	0.686	0.923	0.213	1.938	1.089	1.153
023	324	0.638	0.848	0.169	1.826	1.053	1.066
025	310	0.603	0.836	0.169	1.814	0.962	0.986
027	315	0.640	0.987	0.198	1.931	0.994	0.994
029	329	0.649	0.935	0.193	1.694	0.973	1.044
031	326	0.630	0.888	0.184	1.952	1.058	1.056
033	336	0.684	0.906	0.210	1.908	1.067	1.047
035	322	0.623	0.840	0.190	1.887	0.986	1.064
037	337	0.652	0.915	0.167	1.944	1.122	1.117
039	350	0.763	0.953	0.188	1.998	1.099	1.113
041	319	0.690	0.893	0.186	1.937	1.008	1.014
043	303	0.715	0.861	0.253	1.953	1.042	1.095
045	309	0.622	0.844	0.164	1.733	1.051	0.991
047	312	0.646	0.902	0.190	1.907	1.060	1.030
049	320	0.695	0.871	0.182	1.840	0.956	1.077
051	339	0.674	1.102	0.171	1.928	1.109	1.134
053	343	0.722	0.968	0.223	1.984	1.121	1.150
055	331	0.663	0.833	0.203	1.882	1.035	1.054
057	316	0.690	0.829	0.190	1.798	1.032	1.004
059	317	0.638	0.902	0.188	1.926	1.041	1.029
061							
063	298	0.715	0.821	0.189	1.727	1.032	1.027
065	331	0.679	0.961	0.195	1.636	1.083	1.128
067	304	0.646	0.945	0.206	1.899	1.078	1.115
069	301	0.645	0.858	0.129	1.912	1.045	1.052
071	291	0.628	0.842	0.162	1.927	0.905	0.923
073	292	0.583	0.825	0.157	1.957	0.996	0.981
075	317	0.661	0.836	0.156	1.946	1.000	0.963
077	297	0.592	0.782	0.161	1.970	0.970	0.975
079	346	0.749	0.946	0.234	1.982	1.128	1.146

Table 7. Organ Weights, Male (continued)

Animal #	Adrenal Glands	Liver	Right. Epididymis	Right Testicle	Left Testicle	Left Epididymis
001	0.056	9.088	0.464	1.645	1.592	0.482
003	0.052	9.039	0.524	1.665	1.701	0.548
005	0.044	8.565	0.491	1.651	1.700	0.519
007	0.047	8.370	0.440	1.433	1.544	0.484
009	0.047	8.838	0.454	1.509	1.503	0.485
011	0.053	9.573	0.475	1.561	1.740	0.523
013	0.064	12.586	0.550	1.854	1.847	0.547
015	0.051	10.275	0.470	1.556	1.726	0.468
017	0.045	10.072	0.468	1.538	1.589	0.474
019	0.047	9.985	0.479	1.567	1.646	0.445
021	0.046	9.417	0.506	1.558	1.633	0.521
023	0.047	9.961	0.480	1.495	1.478	0.501
025	0.057	8.938	0.482	1.436	1.465	0.477
027	0.047	9.050	0.459	1.570	1.595	0.459
029	0.044	8.544	0.433	1.394	1.292	0.384
031	0.052	10.323	0.459	1.490	1.622	0.493
033	0.053	10.435	0.475	1.624	1.723	0.529
035	0.052	9.811	0.458	1.553	1.572	0.457
037	0.049	10.369	0.495	1.533	1.639	0.554
039	0.054	10.578	0.512	1.655	1.712	0.482
041	0.060	9.315	0.512	1.540	1.679	0.552
043	0.051	9.257	0.500	1.444	1.544	0.512
045	0.051	8.770	0.479	1.517	1.538	0.516
047	0.036	9.085	0.462	1.469	1.500	0.461
049	0.047	8.504	0.461	1.641	1.680	0.494
051	0.065	11.189	0.492	1.640	1.615	0.479
053	0.048	13.590	0.533	1.618	1.651	0.492
055	0.057	10.308	0.470	1.525	1.584	0.484
057	0.052	9.418	0.500	1.552	1.490	0.455
059	0.055	9.883	0.435	1.492	1.573	0.472
061						
063	0.050	9.381	0.446	1.459	1.315	0.463
065	0.056	9.805	0.462	1.534	1.533	0.496
067	0.049	9.283	0.447	1.556	1.592	0.472
069	0.051	8.545	0.484	1.628	1.531	0.497
071	0.048	8.922	0.442	1.519	1.609	0.446
073	0.046	9.095	0.475	1.515	1.551	0.473
075	0.056	9.739	0.510	1.623	1.634	0.483
077	0.025	8.757	0.507	1.509	1.573	0.480
079	0.048	10.775	0.466	1.513	1.510	0.509

Table 8. Organ Weight to Body Weight Ratio, Male

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
001	318	0.00210	0.00278	0.00067	0.00562	0.00318	0.00328
003	318	0.00210	0.00275	0.00056	0.00627	0.00326	0.00321
005	316	0.00228	0.00267	0.00062	0.00566	0.00308	0.00332
007	298	0.00207	0.00259	0.00076	0.00608	0.00319	0.00321
009	316	0.00188	0.00299	0.00069	0.00619	0.00319	0.00323
011	318	0.00215	0.00256	0.00055	0.00606	0.00297	0.00296
013	405	0.00189	0.00261	0.00062	0.00493	0.00282	0.00275
015	356	0.00197	0.00263	0.00053	0.00537	0.00298	0.00292
017	327	0.00195	0.00271	0.00064	0.00580	0.00324	0.00320
019	328	0.00191	0.00278	0.00056	0.00587	0.00302	0.00309
021	338	0.00203	0.00273	0.00063	0.00573	0.00322	0.00341
023	324	0.00197	0.00262	0.00052	0.00564	0.00325	0.00329
025	310	0.00195	0.00270	0.00055	0.00585	0.00310	0.00318
027	315	0.00203	0.00313	0.00063	0.00613	0.00316	0.00316
029	329	0.00197	0.00284	0.00059	0.00515	0.00296	0.00317
031	326	0.00193	0.00272	0.00056	0.00599	0.00325	0.00324
033	336	0.00204	0.00270	0.00063	0.00568	0.00318	0.00312
035	322	0.00193	0.00261	0.00059	0.00586	0.00306	0.00330
037	337	0.00193	0.00272	0.00050	0.00577	0.00333	0.00331
039	350	0.00218	0.00272	0.00054	0.00571	0.00314	0.00318
041	319	0.00216	0.00280	0.00058	0.00607	0.00316	0.00318
043	303	0.00236	0.00284	0.00083	0.00645	0.00344	0.00361
045	309	0.00201	0.00273	0.00053	0.00561	0.00340	0.00321
047	312	0.00207	0.00289	0.00061	0.00611	0.00340	0.00330
049	320	0.00217	0.00272	0.00057	0.00575	0.00299	0.00337
051	339	0.00199	0.00325	0.00050	0.00569	0.00327	0.00335
053	343	0.00210	0.00282	0.00065	0.00578	0.00327	0.00335
055	331	0.00200	0.00252	0.00061	0.00569	0.00313	0.00318
057	316	0.00218	0.00262	0.00060	0.00569	0.00327	0.00318
059	317	0.00201	0.00285	0.00059	0.00608	0.00328	0.00325
061							
063	298	0.00240	0.00276	0.00063	0.00580	0.00346	0.00345
065	331	0.00205	0.00290	0.00059	0.00494	0.00327	0.00341
067	304	0.00213	0.00311	0.00068	0.00625	0.00355	0.00367
069	301	0.00214	0.00285	0.00043	0.00635	0.00347	0.00350
071	291	0.00216	0.00289	0.00056	0.00662	0.00311	0.00317
073	292	0.00200	0.00283	0.00054	0.00670	0.00341	0.00336
075	317	0.00209	0.00264	0.00049	0.00614	0.00315	0.00304
077	297	0.00199	0.00263	0.00054	0.00663	0.00327	0.00328
079	346	0.00216	0.00273	0.00068	0.00573	0.00326	0.00331

Table 8. Organ Weight to Body Weight Ratio, Male (continued)

Animal #	Adrenal Glands	Liver	Right Epididymis	Right Testicle	Left Testicle	Left Epididymis
001	0.00018	0.02858	0.00146	0.00517	0.00501	0.00152
003	0.00016	0.02842	0.00165	0.00524	0.00535	0.00172
005	0.00014	0.02710	0.00155	0.00522	0.00538	0.00164
007	0.00016	0.02809	0.00148	0.00481	0.00518	0.00162
009	0.00015	0.02797	0.00144	0.00478	0.00476	0.00153
011	0.00017	0.03010	0.00149	0.00491	0.00547	0.00164
013	0.00016	0.03108	0.00136	0.00458	0.00456	0.00135
015	0.00014	0.02886	0.00132	0.00437	0.00485	0.00131
017	0.00014	0.03080	0.00143	0.00470	0.00486	0.00145
019	0.00014	0.03044	0.00146	0.00478	0.00502	0.00136
021	0.00014	0.02786	0.00150	0.00461	0.00483	0.00154
023	0.00015	0.03074	0.00148	0.00461	0.00456	0.00155
025	0.00018	0.02883	0.00155	0.00463	0.00473	0.00154
027	0.00015	0.02873	0.00146	0.00498	0.00506	0.00146
029	0.00013	0.02597	0.00132	0.00424	0.00393	0.00117
031	0.00016	0.03167	0.00141	0.00457	0.00498	0.00151
033	0.00016	0.03106	0.00141	0.00483	0.00513	0.00157
035	0.00016	0.03047	0.00142	0.00482	0.00488	0.00142
037	0.00015	0.03077	0.00147	0.00455	0.00486	0.00164
039	0.00015	0.03022	0.00146	0.00473	0.00489	0.00138
041	0.00019	0.02920	0.00161	0.00483	0.00526	0.00173
043	0.00017	0.03055	0.00165	0.00477	0.00510	0.00169
045	0.00017	0.02838	0.00155	0.00491	0.00498	0.00167
047	0.00012	0.02912	0.00148	0.00471	0.00481	0.00148
049	0.00015	0.02658	0.00144	0.00513	0.00525	0.00154
051	0.00019	0.03301	0.00145	0.00484	0.00476	0.00141
053	0.00014	0.03962	0.00155	0.00472	0.00481	0.00143
055	0.00017	0.03114	0.00142	0.00461	0.00479	0.00146
057	0.00016	0.02980	0.00158	0.00491	0.00472	0.00144
059	0.00017	0.03118	0.00137	0.00471	0.00496	0.00149
061						
063	0.00017	0.03148	0.00150	0.00490	0.00441	0.00155
065	0.00017	0.02962	0.00140	0.00463	0.00463	0.00150
067	0.00016	0.03054	0.00147	0.00512	0.00524	0.00155
069	0.00017	0.02839	0.00161	0.00541	0.00509	0.00165
071	0.00016	0.03066	0.00152	0.00522	0.00553	0.00153
073	0.00016	0.03115	0.00163	0.00519	0.00531	0.00162
075	0.00018	0.03072	0.00161	0.00512	0.00515	0.00152
077	0.00008	0.02948	0.00171	0.00508	0.00530	0.00162
079	0.00014	0.03114	0.00135	0.00437	0.00436	0.00147

Table 9. Organ Weight to Brain Weight Ratio, Male

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
001	178.05	0.373	0.495	0.119	1.000	0.566	0.583
003	159.56	0.335	0.438	0.089	1.000	0.521	0.513
005	176.83	0.402	0.472	0.109	1.000	0.545	0.586
007	164.46	0.340	0.426	0.125	1.000	0.524	0.529
009	161.47	0.303	0.483	0.112	1.000	0.515	0.521
011	164.94	0.354	0.423	0.091	1.000	0.490	0.488
013	202.80	0.383	0.530	0.126	1.000	0.572	0.558
015	186.10	0.366	0.489	0.099	1.000	0.554	0.544
017	172.56	0.337	0.468	0.110	1.000	0.558	0.552
019	170.39	0.325	0.473	0.095	1.000	0.514	0.526
021	174.41	0.354	0.476	0.110	1.000	0.562	0.595
023	177.44	0.349	0.464	0.093	1.000	0.577	0.584
025	170.89	0.332	0.461	0.093	1.000	0.530	0.544
027	163.13	0.331	0.511	0.103	1.000	0.515	0.515
029	194.21	0.383	0.552	0.114	1.000	0.574	0.616
031	167.01	0.323	0.455	0.094	1.000	0.542	0.541
033	176.10	0.358	0.475	0.110	1.000	0.559	0.549
035	170.64	0.330	0.445	0.101	1.000	0.523	0.564
037	173.35	0.335	0.471	0.086	1.000	0.577	0.575
039	175.18	0.382	0.477	0.094	1.000	0.550	0.557
041	164.69	0.356	0.461	0.096	1.000	0.520	0.523
043	155.15	0.366	0.441	0.130	1.000	0.534	0.561
045	178.30	0.359	0.487	0.095	1.000	0.606	0.572
047	163.61	0.339	0.473	0.100	1.000	0.556	0.540
049	173.91	0.378	0.473	0.099	1.000	0.520	0.585
051	175.83	0.350	0.572	0.089	1.000	0.575	0.588
053	172.88	0.364	0.488	0.112	1.000	0.565	0.580
055	175.88	0.352	0.443	0.108	1.000	0.550	0.560
057	175.75	0.384	0.461	0.106	1.000	0.574	0.558
059	164.59	0.331	0.468	0.098	1.000	0.540	0.534
061							
063	172.55	0.414	0.475	0.109	1.000	0.598	0.595
065	202.32	0.415	0.587	0.119	1.000	0.662	0.689
067	160.08	0.340	0.498	0.108	1.000	0.568	0.587
069	157.43	0.337	0.449	0.067	1.000	0.547	0.550
071	151.01	0.326	0.437	0.084	1.000	0.470	0.479
073	149.21	0.298	0.422	0.080	1.000	0.509	0.501
075	162.90	0.340	0.430	0.080	1.000	0.514	0.495
077	150.76	0.301	0.397	0.082	1.000	0.492	0.495
079	174.57	0.378	0.477	0.118	1.000	0.569	0.578

Table 9. Organ Weight to Brain Weight Ratio, Male (continued)

Animal #	Adrenal Glands	Liver	Right Epididymis	Right Testicle	Left Testicle	Left Epididymis
001	0.031	5.088	0.260	0.921	0.891	0.270
003	0.026	4.535	0.263	0.835	0.853	0.275
005	0.025	4.793	0.275	0.924	0.951	0.290
007	0.026	4.619	0.243	0.791	0.852	0.267
009	0.024	4.516	0.232	0.771	0.768	0.248
011	0.027	4.965	0.246	0.810	0.902	0.271
013	0.032	6.302	0.275	0.928	0.925	0.274
015	0.027	5.371	0.246	0.813	0.902	0.245
017	0.024	5.315	0.247	0.812	0.839	0.250
019	0.024	5.187	0.249	0.814	0.855	0.231
021	0.024	4.859	0.261	0.804	0.843	0.269
023	0.026	5.455	0.263	0.819	0.809	0.274
025	0.031	4.927	0.266	0.792	0.808	0.263
027	0.024	4.687	0.238	0.813	0.826	0.238
029	0.026	5.044	0.256	0.823	0.763	0.227
031	0.027	5.288	0.235	0.763	0.831	0.253
033	0.028	5.469	0.249	0.851	0.903	0.277
035	0.028	5.199	0.243	0.823	0.833	0.242
037	0.025	5.334	0.255	0.789	0.843	0.285
039	0.027	5.294	0.256	0.828	0.857	0.241
041	0.031	4.809	0.264	0.795	0.867	0.285
043	0.026	4.740	0.256	0.739	0.791	0.262
045	0.029	5.061	0.276	0.875	0.887	0.298
047	0.019	4.764	0.242	0.770	0.787	0.242
049	0.026	4.622	0.251	0.892	0.913	0.268
051	0.034	5.803	0.255	0.851	0.838	0.248
053	0.024	6.850	0.269	0.816	0.832	0.248
055	0.030	5.477	0.250	0.810	0.842	0.257
057	0.029	5.238	0.278	0.863	0.829	0.253
059	0.029	5.131	0.226	0.775	0.817	0.245
061						
063	0.029	5.432	0.258	0.845	0.761	0.268
065	0.034	5.993	0.282	0.938	0.937	0.303
067	0.026	4.888	0.235	0.819	0.838	0.249
069	0.027	4.469	0.253	0.851	0.801	0.260
071	0.025	4.630	0.229	0.788	0.835	0.231
073	0.024	4.647	0.243	0.774	0.793	0.242
075	0.029	5.005	0.262	0.834	0.840	0.248
077	0.013	4.445	0.257	0.766	0.798	0.244
079	0.024	5.436	0.235	0.763	0.762	0.257

Table 10. Organ Weights, Female (all weights in grams)

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
002	179	0.462	0.558	0.188	1.742	0.577	0.588
004	183	0.513	0.594	0.182	1.651	0.624	0.638
006	202	0.505	0.649	0.204	1.807	0.646	0.690
066	164	0.408	0.581	0.162	1.740	0.552	0.544
010	169	0.435	0.546	0.168	1.747	0.592	0.604
012	174	0.407	0.528	0.180	1.792	0.612	0.596
014	184	0.489	0.690	0.989	1.821	0.644	0.652
016	171	0.400	0.595	0.158	1.761	0.556	0.595
018	170	0.421	0.599	0.157	1.816	0.548	0.628
020	166	0.432	0.597	0.167	1.736	0.584	0.551
022	187	0.471	0.616	0.195	1.795	0.642	0.669
024	179	0.474	0.958	0.215	1.832	0.595	0.630
026	186	0.512	0.665	0.197	1.713	0.675	0.693
028	177	0.486	0.615	0.175	1.673	0.662	0.645
030	190	0.485	0.664	0.173	1.739	0.635	0.658
032	188	0.457	0.594	0.195	1.676	0.650	0.631
034	176	0.429	0.598	0.158	1.824	0.611	0.599
036	181	0.447	0.663	0.155	1.830	0.605	0.643
038	168	0.461	0.576	0.195	1.680	0.568	0.595
040	191	0.469	0.627	0.182	1.808	0.631	0.619
042	190	0.526	0.572	0.213	1.805	0.642	0.663
044	183	0.527	0.662	0.194	1.578	0.635	0.704
046							
048	177	0.503	0.612	0.165	1.764	0.633	0.638
050	186	0.465	0.731	0.188	1.823	0.649	0.687
052	186	0.531	0.776	0.169	1.815	0.668	0.701
054	176	0.461	0.709	0.187	1.711	0.625	0.586
056	175	0.473	0.665	0.169	1.772	0.620	0.603
058	191	0.480	0.665	0.185	1.836	0.630	0.681
060	166	0.505	0.619	0.150	1.795	0.566	0.638
062	160	0.457	0.543	0.200	1.585	0.582	0.616
064	176	0.491	0.607	0.195	1.733	0.658	0.673
008	162	0.395	0.593	0.119	1.666	0.595	0.626
068	170	0.423	0.618	0.178	1.774	0.622	0.593
070	168	0.417	0.669	0.131	1.776	0.619	0.632
072	167	0.396	0.552	0.149	1.583	0.593	0.614
074	188	0.517	0.694	0.191	1.782	0.721	0.699
076	179	0.427	0.561	0.165	1.809	0.686	0.652
078	166	0.434	0.598	0.155	1.751	0.574	0.624
080	172	0.429	0.639	0.169	1.815	0.585	0.599

Table 10. Organ Weights, Female (continued)

Animal #	Adrenal Glands	Liver	Uterus	Ovaries
002	0.063	4.990	0.647	0.117
004	0.055	4.750	0.767	0.107
006	0.054	5.127	0.538	0.111
066	0.053	4.020	0.962	0.082
010	0.055	4.562	0.516	0.101
012	0.055	4.279	0.602	0.100
014	0.058	4.771	0.582	0.098
016	0.056	4.323	1.420	0.099
018	0.056	4.959	0.392	0.090
020	0.058	4.781	1.192	0.103
022	0.057	4.936	0.747	0.081
024	0.052	4.961	0.473	0.088
026	0.060	5.787	0.575	0.103
028	0.056	4.659	0.819	0.110
030	0.057	4.844	0.594	0.115
032	0.056	4.980	0.469	0.095
034	0.049	4.573	0.391	0.096
036	0.055	4.756	0.614	0.092
038	0.055	4.586	1.042	0.096
040	0.051	4.923	1.074	0.110
042	0.060	5.158	0.797	0.116
044	0.053	5.229	0.540	0.106
046				
048	0.062	4.927	1.168	0.075
050	0.059	5.443	0.583	0.105
052	0.059	5.265	0.786	0.109
054	0.060	5.282	0.540	0.095
056	0.049	4.959	0.524	0.104
058	0.052	5.210	0.646	0.114
060	0.055	4.999	0.470	0.080
062	0.054	4.614	1.229	0.089
064	0.065	5.092	0.488	0.100
008	0.064	4.547	0.331	0.048
068	0.048	4.696	0.811	0.104
070	0.056	5.176	0.436	0.111
072	0.064	4.687	0.454	0.087
074	0.066	5.534	1.029	0.105
076	0.058	4.826	0.379	0.091
078	0.055	4.528	0.436	0.073
080	0.059	4.682	0.771	0.096

Table 11. Organ Weight to Body Weight Ratios, Female

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
002	1.0	0.00258	0.00312	0.00105	0.00973	0.00322	0.00328
004	1.0	0.00280	0.00325	0.00099	0.00902	0.00341	0.00349
006	1.0	0.00250	0.00321	0.00101	0.00895	0.00320	0.00342
066	1.0	0.00249	0.00354	0.00099	0.01061	0.00337	0.00332
010	1.0	0.00257	0.00323	0.00099	0.01034	0.00350	0.00357
012	1.0	0.00234	0.00303	0.00103	0.01030	0.00352	0.00343
014	1.0	0.00266	0.00375	0.00538	0.00990	0.00350	0.00354
016	1.0	0.00234	0.00348	0.00092	0.01030	0.00325	0.00348
018	1.0	0.00248	0.00352	0.00092	0.01068	0.00322	0.00369
020	1.0	0.00260	0.00360	0.00101	0.01046	0.00352	0.00332
022	1.0	0.00252	0.00329	0.00104	0.00960	0.00343	0.00358
024	1.0	0.00265	0.00535	0.00120	0.01023	0.00332	0.00352
026	1.0	0.00275	0.00358	0.00106	0.00921	0.00363	0.00373
028	1.0	0.00275	0.00347	0.00099	0.00945	0.00374	0.00364
030	1.0	0.00255	0.00349	0.00091	0.00915	0.00334	0.00346
032	1.0	0.00243	0.00316	0.00104	0.00891	0.00346	0.00336
034	1.0	0.00244	0.00340	0.00090	0.01036	0.00347	0.00340
036	1.0	0.00247	0.00366	0.00086	0.01011	0.00334	0.00355
038	1.0	0.00274	0.00343	0.00116	0.01000	0.00338	0.00354
040	1.0	0.00246	0.00328	0.00095	0.00947	0.00330	0.00324
042	1.0	0.00277	0.00301	0.00112	0.00950	0.00338	0.00349
044	1.0	0.00288	0.00362	0.00106	0.00862	0.00347	0.00385
046	1.0						
048	1.0	0.00284	0.00346	0.00093	0.00997	0.00358	0.00360
050	1.0	0.00250	0.00393	0.00101	0.00980	0.00349	0.00369
052	1.0	0.00285	0.00417	0.00091	0.00976	0.00359	0.00377
054	1.0	0.00262	0.00403	0.00106	0.00972	0.00355	0.00333
056	1.0	0.00270	0.00380	0.00097	0.01013	0.00354	0.00345
058	1.0	0.00251	0.00348	0.00097	0.00961	0.00330	0.00357
060	1.0	0.00304	0.00373	0.00090	0.01081	0.00341	0.00384
062	1.0	0.00286	0.00339	0.00125	0.00991	0.00364	0.00385
064	1.0	0.00279	0.00345	0.00111	0.00985	0.00374	0.00382
008	1.0	0.00244	0.00366	0.00073	0.01028	0.00367	0.00386
068	1.0	0.00249	0.00364	0.00105	0.01044	0.00366	0.00349
070	1.0	0.00248	0.00398	0.00078	0.01057	0.00368	0.00376
072	1.0	0.00237	0.00331	0.00089	0.00948	0.00355	0.00368
074	1.0	0.00275	0.00369	0.00102	0.00948	0.00384	0.00372
076	1.0	0.00239	0.00313	0.00092	0.01011	0.00383	0.00364
078	1.0	0.00261	0.00360	0.00093	0.01055	0.00346	0.00376
080	1.0	0.00249	0.00372	0.00098	0.01055	0.00340	0.00348

Table 11. Organ Weight to Body Weight Ratios, Female (continued)

Animal #	Adrenal Glands	Liver	Uterus	Ovaries
002	0.00035	0.02788	0.00361	0.00065
004	0.00030	0.02596	0.00419	0.00058
006	0.00027	0.02538	0.00266	0.00055
066	0.00032	0.02451	0.00587	0.00050
010	0.00033	0.02699	0.00305	0.00060
012	0.00032	0.02459	0.00346	0.00057
014	0.00032	0.02593	0.00316	0.00053
016	0.00033	0.02528	0.00830	0.00058
018	0.00033	0.02917	0.00231	0.00053
020	0.00035	0.02880	0.00718	0.00062
022	0.00030	0.02640	0.00399	0.00043
024	0.00029	0.02772	0.00264	0.00049
026	0.00032	0.03111	0.00309	0.00055
028	0.00032	0.02632	0.00463	0.00062
030	0.00030	0.02549	0.00313	0.00061
032	0.00030	0.02649	0.00249	0.00051
034	0.00028	0.02598	0.00222	0.00055
036	0.00030	0.02628	0.00339	0.00051
038	0.00033	0.02730	0.00620	0.00057
040	0.00027	0.02577	0.00562	0.00058
042	0.00032	0.02715	0.00419	0.00061
044	0.00029	0.02857	0.00295	0.00058
046				
048	0.00035	0.02784	0.00660	0.00042
050	0.00032	0.02926	0.00313	0.00056
052	0.00032	0.02831	0.00423	0.00059
054	0.00034	0.03001	0.00307	0.00054
056	0.00028	0.02834	0.00299	0.00059
058	0.00027	0.02728	0.00338	0.00060
060	0.00033	0.03011	0.00283	0.00048
062	0.00034	0.02884	0.00768	0.00056
064	0.00037	0.02893	0.00277	0.00057
008	0.00040	0.02807	0.00204	0.00030
068	0.00028	0.02762	0.00477	0.00061
070	0.00033	0.03081	0.00260	0.00066
072	0.00038	0.02807	0.00272	0.00052
074	0.00035	0.02944	0.00547	0.00056
076	0.00032	0.02696	0.00212	0.00051
078	0.00033	0.02728	0.00263	0.00044
080	0.00034	0.02722	0.00448	0.00056

Table 12. Organ Weight to Brain Weight Ratios, Female

Animal #	Body Weight	Spleen	Heart	Thymus	Brain	Right Kidney	Left Kidney
002	102.8	0.265	0.320	0.108	1.000	0.331	0.338
004	110.8	0.311	0.360	0.110	1.000	0.378	0.386
006	111.8	0.279	0.359	0.113	1.000	0.357	0.382
066	94.3	0.234	0.334	0.093	1.000	0.317	0.313
010	96.7	0.249	0.313	0.096	1.000	0.339	0.346
012	97.1	0.227	0.295	0.100	1.000	0.342	0.333
014	101.0	0.269	0.379	0.543	1.000	0.354	0.358
016	97.1	0.227	0.338	0.090	1.000	0.316	0.338
018	93.6	0.232	0.330	0.086	1.000	0.302	0.346
020	95.6	0.249	0.344	0.096	1.000	0.336	0.317
022	104.2	0.262	0.343	0.109	1.000	0.358	0.373
024	97.7	0.259	0.523	0.117	1.000	0.325	0.344
026	108.6	0.299	0.388	0.115	1.000	0.394	0.405
028	105.8	0.290	0.368	0.105	1.000	0.396	0.386
030	109.3	0.279	0.382	0.099	1.000	0.365	0.378
032	112.2	0.273	0.354	0.116	1.000	0.388	0.376
034	96.5	0.235	0.328	0.087	1.000	0.335	0.328
036	98.9	0.244	0.362	0.085	1.000	0.331	0.351
038	100.0	0.274	0.343	0.116	1.000	0.338	0.354
040	105.6	0.259	0.347	0.101	1.000	0.349	0.342
042	105.3	0.291	0.317	0.118	1.000	0.356	0.367
044	116.0	0.334	0.420	0.123	1.000	0.402	0.446
046							
048	100.3	0.285	0.347	0.094	1.000	0.359	0.362
050	102.0	0.255	0.401	0.103	1.000	0.356	0.377
052	102.5	0.293	0.428	0.093	1.000	0.368	0.386
054	102.9	0.269	0.414	0.109	1.000	0.365	0.342
056	98.8	0.267	0.375	0.095	1.000	0.350	0.340
058	104.0	0.261	0.362	0.101	1.000	0.343	0.371
060	92.5	0.281	0.345	0.084	1.000	0.315	0.355
062	100.9	0.288	0.343	0.126	1.000	0.367	0.389
064	101.6	0.283	0.350	0.113	1.000	0.380	0.388
008	97.2	0.237	0.356	0.071	1.000	0.357	0.376
068	95.8	0.238	0.348	0.100	1.000	0.351	0.334
070	94.6	0.235	0.377	0.074	1.000	0.349	0.356
072	105.5	0.250	0.349	0.094	1.000	0.375	0.388
074	105.5	0.290	0.389	0.107	1.000	0.405	0.392
076	98.9	0.236	0.310	0.091	1.000	0.379	0.360
078	94.8	0.248	0.342	0.089	1.000	0.328	0.356
080	94.8	0.236	0.352	0.093	1.000	0.322	0.330

Table 12. Organ Weight to Brain Weight Ratios, Female (continued)

Animal #	Adrenal Glands	Liver	Uterus	Ovaries
002	0.036	2.865	0.371	0.067
004	0.033	2.877	0.465	0.065
006	0.030	2.837	0.298	0.061
066	0.030	2.310	0.553	0.047
010	0.031	2.611	0.295	0.058
012	0.031	2.388	0.336	0.056
014	0.032	2.620	0.320	0.054
016	0.032	2.455	0.806	0.056
018	0.031	2.731	0.216	0.050
020	0.033	2.754	0.687	0.059
022	0.032	2.750	0.416	0.045
024	0.028	2.708	0.258	0.048
026	0.035	3.378	0.336	0.060
028	0.033	2.785	0.490	0.066
030	0.033	2.786	0.342	0.066
032	0.033	2.971	0.280	0.057
034	0.027	2.507	0.214	0.053
036	0.030	2.599	0.336	0.050
038	0.033	2.730	0.620	0.057
040	0.028	2.723	0.594	0.061
042	0.033	2.858	0.442	0.064
044	0.034	3.314	0.342	0.067
046				
048	0.035	2.793	0.662	0.043
050	0.032	2.986	0.320	0.058
052	0.033	2.901	0.433	0.060
054	0.035	3.087	0.316	0.056
056	0.028	2.799	0.296	0.059
058	0.028	2.838	0.352	0.062
060	0.031	2.785	0.262	0.045
062	0.034	2.911	0.775	0.056
064	0.038	2.938	0.282	0.058
008	0.038	2.729	0.199	0.029
068	0.027	2.647	0.457	0.059
070	0.032	2.914	0.245	0.063
072	0.040	2.961	0.287	0.055
074	0.037	3.105	0.577	0.059
076	0.032	2.668	0.210	0.050
078	0.031	2.586	0.249	0.042
080	0.033	2.580	0.425	0.053

Table 13. Clinical Observations

Animal Number	Clinical Observation	Animal Number	Clinical Observation
1	NAD	2	NAD
3	NAD	4	NAD
5	NAD	6	NAD
7	NAD	8	NAD
9	NAD	10	NAD
11	NAD	12	NAD
13	SD13: Reddened Eye; SD14: OK	14	NAD
15	NAD	16	NAD
17	NAD	18	NAD
19	NAD	20	NAD
21	NAD	22	NAD
23	NAD	24	NAD
25	NAD	26	NAD
27	NAD	28	NAD
29	NAD	30	NAD
31	NAD	32	NAD
33	NAD	34	NAD
35	NAD	36	NAD
37	NAD	38	NAD
39	NAD	40	NAD
41	NAD	42	NAD
43	NAD	44	NAD
45	NAD	46	SD103: Accidental death
47	NAD	48	NAD
49	NAD	50	NAD
51	SD62: Reddish discharge, nose	52	NAD
53	NAD	54	NAD
55	NAD	56	NAD
57	NAD	58	NAD
59	NAD	60	NAD
61	SD103: Accidental death	62	NAD
63	NAD	64	SD93: Tail had red sores/cuts
65	NAD	66	SD64: Head tilted to left
67	NAD	68	NAD
69	NAD	70	NAD
71	NAD	72	NAD
73	NAD	74	NAD
75	NAD	76	NAD
77	NAD	78	NAD
79	NAD	80	NAD

Table 14. Gross Pathology Observations at Necropsy

Animal #	Dose Group	Sex	Start date	Necropsy Date	Disposition	Gross Lesions	Notes at Necropsy
001	C	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
003	C	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
005	C	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
007	C	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
009	C	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
011	C	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		Unusual INR
013	C	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
015	C	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
017	C	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
019	C	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
002	C	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
004	C	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
006	C	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
066	C	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
010	C	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
012	C	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
014	C	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
016	C	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
018	C	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
020	C	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		

Unusual INR: INR = International Normalized Ratio. This refers to a reading on the instrument measuring blood clotting time.

Table 14. Gross Pathology Observations at Necropsy (continued).

Animal #	Dose Group	Sex	Start date	Necropsy Date	Disposition	Gross Lesions	Notes at Necropsy
021	L	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
023	L	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
025	L	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
027	L	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
029	L	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
031	L	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
033	L	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
035	L	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
037	L	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
039	L	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
022	L	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
024	L	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
026	L	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
028	L	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
030	L	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
032	L	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
034	L	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
036	L	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
038	L	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
040	L	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		

Table 14. Gross Observations at Necropsy (continued).

Animal #	Dose Group	Sex	Start date	Necropsy Date	Disposition	Gross Lesions	Notes at Necropsy
041	M	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
043	M	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
045	M	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
047	M	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
049	M	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
051	M	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
053	M	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
055	M	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy	.	String was caught on left lobe of lung
057	M	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
059	M	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
042	M	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
044	M	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
046	M	F	5/11/2011	21-Aug-11	Found Dead		
048	M	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		Congested lymph node in cassette. No spine in jar
050	M	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
052	M	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
054	M	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		Right femur placed in jar and marrow pulled from left femur
056	M	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
058	M	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
060	M	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		

Table 14. Gross Pathology Observations at Necropsy (continued).

Animal #	Dose Group	Sex	Start date	Necropsy Date	Disposition	Gross Lesions	Notes at Necropsy
061	H	M	5/11/2011	21-Aug-11	Found Dead		
063	H	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
065	H	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
067	H	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
069	H	M	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
071	H	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
073	H	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
075	H	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
077	H	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy	Only (1) adrenal found (right).	
079	H	M	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
062	H	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy	Hydro-ureter	
064	H	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
008	H	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
068	H	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
070	H	F	5/11/2011	24-Aug-11	Euthanasia/Necropsy		
072	H	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
074	H	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
076	H	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
078	H	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		
080	H	F	5/12/2011	25-Aug-11	Euthanasia/Necropsy		

APPENDIX D. NEUROTOXICITY TESTING (MOTOR ACTIVITY AND FOB) IN F344 RATS EXPOSED TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (*Rattus norvegicus*) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

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Introduction

Civilian and military personnel may be occupationally exposed to hydrocarbon fuels such as gasoline, jet fuel, diesel fuel, kerosene, and others. These exposures may occur to raw fuel vapor or aerosol droplets, or to fuel combustion exhaust on an acute or chronic basis, by dermal, respiratory inhalation, or ingestion routes (Ritchie *et al.*, 2001). Petroleum-based fuels typically are a complex mixture of aliphatic and aromatic hydrocarbons, which may include known neurotoxicants. A number of published studies have documented neurotoxicity in humans or animals arising from acute, subchronic, or chronic exposure to hydrocarbon fuels (Ritchie *et al.*, 2001). Standard fuels such as JP-8 jet fuel have been studied for neurotoxicity (Rossi *et al.*, 2001), but new synthetic or bio-derived fuels are being developed as a substitute or replacement for petroleum-derived JP-8 for military use by the US armed forces. A Hydrotreated Renewable Jet (HRJ) fuel produced from an extract of the camelina plant is one such fuel. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential general toxicity and neurotoxicity of HRJ fuel. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of HRJ Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a greater than 90-day period, at concentrations of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. In order to determine if exposure to HRJ fuel caused any neurotoxicity, animals were assessed for motor activity (MA) and a functional observational battery (FOB) was conducted towards the end of exposures.

Materials and Methods

Study Design: This study followed methodologies in the USEPA Guideline OPPTS 870.3465 Health Effects Test Guidelines, 90-Day Inhalation Toxicity. Formal observation of animal behavior as described below for motor activity and functional observational battery are specified endpoints for this study. The U.S. EPA Health Effects Test Guideline OPPTS 870.6200 "Neurotoxicity Screening Battery" describes the specific observations made. These endpoints are intended to identify unusual or abnormal behavior that may indicate potential neurotoxic effects caused by the inhalation of HRJ fuel.

Animals and Exposures: F344 rats were used for this study as the neurotoxicology guidelines specifically state that rats or mice should be used and they are a common representative species for toxicity studies. These animals (10/sex/exposure concentration) were also evaluated for inhalation toxicity and reproductive toxicity as described in the main body of the report. Details concerning the animals used on this study and their husbandry may be found in the main report. Animals were assigned to two different replicate groups for exposure scheduling. The replicates were staggered by a day when beginning the exposures, in order to conduct the MA, FOB, and necropsies over two-day periods. The MA was conducted after about 64 days of exposure, and the FOB after 68 days of exposure. A description of the generation and exposure system, the characterization of the exposure atmosphere, and environmental conditions during exposures are provided in the main report.

Neurobehavioral Procedures

Motor Activity (MA): Gross locomotor movements and exploratory behavior were evaluated in the animals using a photobeam activity system and software (PAS) (SDI, San Diego, CA). Animals were individually placed in a 16" (W) x 16" (D) x 15" (H) open field surrounded by frame with 16 x 16 photobeam array. One frame was placed at ground level to detect horizontal movement and to differentiate small (stereotypic) movements from large movements, and a second frame was elevated above the ground level frame to detect vertical rears. As the test animal moved through the photobeam array, beam breaks were automatically recorded using the PAS software. The apparatuses were located in a room with white noise generated at 73dB to mask ambient room levels of ~70dB and low illuminating light set at 30 lux. To begin the test, animals were placed in the center of the open field and left uninterrupted for the duration of a 1 hour test session. A computer system automatically recorded all beam breaks. The following dependent measures were automatically recorded: distance traveled (cm), time spent active/ time spent resting (sec), average speed (cm/sec), number of fine beam breaks (stereotypical), total number of rears, percentage of time in center vs. perimeter, and total activity habituation over six 10 minute blocks. Between tests, all fecal boli were removed and the open fields were wiped down with a solution of 10% ethanol to remove any olfactory cues that may have been left by the previous animal.

Functional Observational Battery (FOB): On a non-exposure day following 3 consecutive days of exposures, animals from one replicate group were moved to a neurotoxicology laboratory. The second replicate group was transferred the next day. A functional observational battery (FOB) consisting of non-invasive procedures designed to evaluate and document the absence or presence (or severity if appropriate) of a predetermined set of behavioral and clinical signs was performed. Observations are made: 1) while the rat was in an observation cage, 2) during removal of the rat from the observation cage, 3) while the rat was being held and examined for clinical observations, 4) as the animal moved freely about the open field, and 5) during manipulative tests. The observations proceeded from the least to most manipulative tests to reduce the influence of handling on the rat's behavior. Efforts were made to control conditions that could affect behavior including sound level, temperature, humidity, lighting, odors, time of day and environmental distractions.

The FOB included the following observations, made and recorded in accordance with the test facility SOP:

- In cage observations: Posture, tremors and spasms, and palpebral closure.
- Observations during removal from cage and handling: Reactivity to handling, muscle tone, lacrimation, salivation, fur appearance, facial crust, breathing pattern, and other clinical signs.
- Open Field Observations: Arousal and activity level, gait, body position, vocalization, tremor, spasm, unusual behaviors, urine and defecation count.
- Manipulative Observations:
    - Approach response: response to a blunt object approaching and stopping before the animal's nose.
    - Acoustic response: response to a hidden metallic click.
    - Tail pinch response: response to a pinch of the tail.
    - Visual placement: response of forelimb to grasp for a surface while being held by the observer.
    - Surface righting: righting response to being turned and briefly held on its back.
    - Hind leg splay: response to being dropped approximately 30 cm (12 inches). Hind legs are painted to mark the location of the hind legs upon landing. This test is not

done if animal is judged too weak to support its weight when dropped or if righting response is not displayed.
- Grip strength: force necessary to break the animal's grip on a wire mesh.
- Pupil reflex: pupil response to light.
- Body weight (if not weighed earlier on the day of the FOB).

Statistical analysis: For neurobehavioral assessments, the data for quantitative, continuous variables were compared for the exposure and control groups by tests for homogeneity of variance, 2-way fixed effects (dose and sex) analysis of variance (ANOVA), and Dunnett's multiple comparison procedure for significant ANOVAs. If the ANOVA indicated statistical significance among experimental groups, the Dunnett's test was used to delineate which groups differ from the control group. A natural log transformation of the data was used if the Levene's test indicated that the data was non-homogeneous. In the event that the Levene's test on the transformed data indicated non-homogeneous data, a Kruskal-Wallis or Wilcoxon Rank-sum test was used. When assumptions for parametric ANOVA were not met, nonparametric procedures were used. Additional exposure group comparisons of various test session activities were also performed. Incidence data was compared using the appropriate statistical test, generally Fisher's Exact test. Incidence data for selected FOB endpoints with ordered severity scores was analyzed for group differences using appropriate measures of association. Statistical analyses were performed using SigmaPlot or other statistical programs, as deemed appropriate. The probability value of less than 0.05 was used as the critical level of significance for each statistical test, except that the critical level of significance for Levene's test for homogeneity of variance was less than 0.01. All other uncorrected probability values of less than 0.05 are listed in the report.

Results

During this study, adult male and female rats were exposed by whole body inhalation to air (control), low, medium or high HRJ fuel for 6 hr/day 5 d/wk over more than 90 days. All exposure groups were evaluated for neurobehavioral effects using a small battery of neurobehavioral tests that comprise the motor activity (MA) and functional observational battery (FOB) conducted in our laboratory. No significant dose related activity or FOB effects were found for males or females under any exposure conditions (Table 1).

Motor Activity: No significant differences between exposure groups were detected for the males or females for any of the motor activity measurements. Analyses for total distance, activity time, average speed, total rears, percentage of time in center vs. perimeter, and habituation activity over 60 minutes provided no differences between exposure groups. Fig. 1 and Table nn shows the total activity habituation (beam breaks) in 10 minute blocks over 60 minutes for the males. There was an expected main effect for block time with decreased activity over the 60 minutes but no main effect for exposure group or interaction for exposure group x block . Fig. 2 and Table nn shows the total activity habituation (beam breaks) in 10 minute blocks over 60 minutes for the females. There was an expected main effect for block time with decreased activity over the 60 minutes but no main effect for exposure group or interaction for exposure group x block. Total activity times (in seconds out of 3600) are shown in Table 1 for both males and females.

FOB: For all functional observations including cage side, open field and manipulation tests, no dose related effects were reported for either males or females. Chi-square statistics (Chi-square= 12.311 with 12 degrees of freedom. P = 0.421) for proportion of observations between

exposure groups for types of facial crusting are shown in Figure 3 (Males) and Figure 4 (Female). Results for rears, stereotypical grooming bouts, hind limb splay, and forelimb grip strength are show in Table 1 for both males and females.

References

Ritchie GD, Still KR, Alexander WK, Nordholm AF, Wilson CL, Rossi J 3rd, Mattie DR. A review of the neurotoxicity risk of selected hydrocarbon fuels. J Toxicol Environ Health B Crit Rev. (2001) Jul-Sep;4(3):223-312.

Rossi, J 3rd, Nordholm AF, Carpenter RL, Ritchie GD, Malcomb W. Effects of repeated exposure of rats to JP-5 or JP-8 jet fuel vapor on neurobehavioral capacity and neurotransmitter levels. J Toxicol Environ Health A. (2001) 63:397-428.

Table 1: Summary of Results from Neurobehavioral Tests for HRJ Exposures

	HRJ Fuel Exposure Atmospheres Target Concentration			
Neurobehavioral Test	**Control**	**Low (200 mg/m^3)**	**Intermediate (700 mg/m^3)**	**High (2000 mg/m^3)**
Male MA Total Activity (sec) (out of 3600 sec)	1877.77±114.58	1960.1±130.23	2152.51±78.12	2007.04±98.14
Male FOB Rears (Number)	16.7±1.38	17.5±2.00	20.2±1.90	17.1±1.52
Male FOB Stereo Grooming (Number)	3.2±0.39	3.3±0.47	3.9±0.62	4.0±0.49
Male FOB Hind Splay (cm)	6.17±0.23	5.37±0.28	5.27±0.25	5.37±0.15
Male FOB Forelimb Grip (Kg)	0.55±0.05	0.45±0.03	0.44±0.05	0.57±0.06
Female MA Total Activity (sec) (out of 3600 sec)	1864.13±44.25	1969.62±87.96	1817.02±109.76	1946±101.74
Female FOB Rears (Number)	20.20±1.50	19.4±1.22	19.2±0.89	20.3±1.22
Female FOB Stereo Grooming (Number)	4.6±0.50	5.2±0.47	4.6±0.48	4.3±0.37
Female FOB Hind Splay (cm)	4.44±0.33	4.76±0.32	4.92±0.33	5.12±0.35
Female FOB Forelimb Grip (Kg)	0.36±0.04	0.36±0.03	0.33±0.02	0.31±0.02

Table 2. Habituation Motor Activity for Male Rats Exposed to HRJ Fuel

Interval	Control	Low (200 mg/m^3)	Intermediate (700 mg/m^3)	High (2000 mg/m^3)
1 (0-10)	739.0 (71.699)	742.2 (72.579)	669.2 (70.406)	618.0 (48.845)
2 (11-20)	418.1 (62.404)	429.3 (55.029)	466.1 (42.040)	354.4 (32.401)
3 (21-30)	330.2 (69.376)	254.3 (40.846)	310.9 (30.708)	288.7 (29.741)
4 (31-40)	287.0 (35.636)	211.4 (49.672)	207.2 (24.347)	186.1 (26.160)
5(41-50)	144.8 (31.030)	240.9 (44.205)	166.9 (35.024)	202.4 (25.053)
6 (51-60)	175.5 (47.593)	189.1 (42.437)	200.5 (33.296)	146.3 (26.606)

Mean ± SEM, beam breaks per interval

Table 3. Habituation Motor Activity for Female Rats Exposed to HRJ Fuel

Interval	Control	Low (200 mg/m^3)	Intermediate (700 mg/m^3)	High (2000 mg/m^3)
1 (0-10)	354.9 (26.495)	534.4 (55.997)	517.2 (51.136)	540.0 (72.415)
2 (11-20)	259.8 (23.862)	336.0 (41.406)	297.0 (40.551)	358.6 (45.807)
3 (21-30)	234.3 (31.920)	266.9 (37.963)	207.5 (19.993)	296.8 (45.732)
4 (31-40)	212.3 (26.162)	246.5 (46.609)	219.4 (48.774)	240.1 (39.404)
5(41-50)	141.2 (18.640)	188.4 (38.974)	170.7 (39.818)	178.4 (20.924)
6 (51-60)	129.4 (15.531)	175.0 (33.901)	167.6 (24.365)	156.1 (23.103)

Mean ± SEM, beam breaks per interval

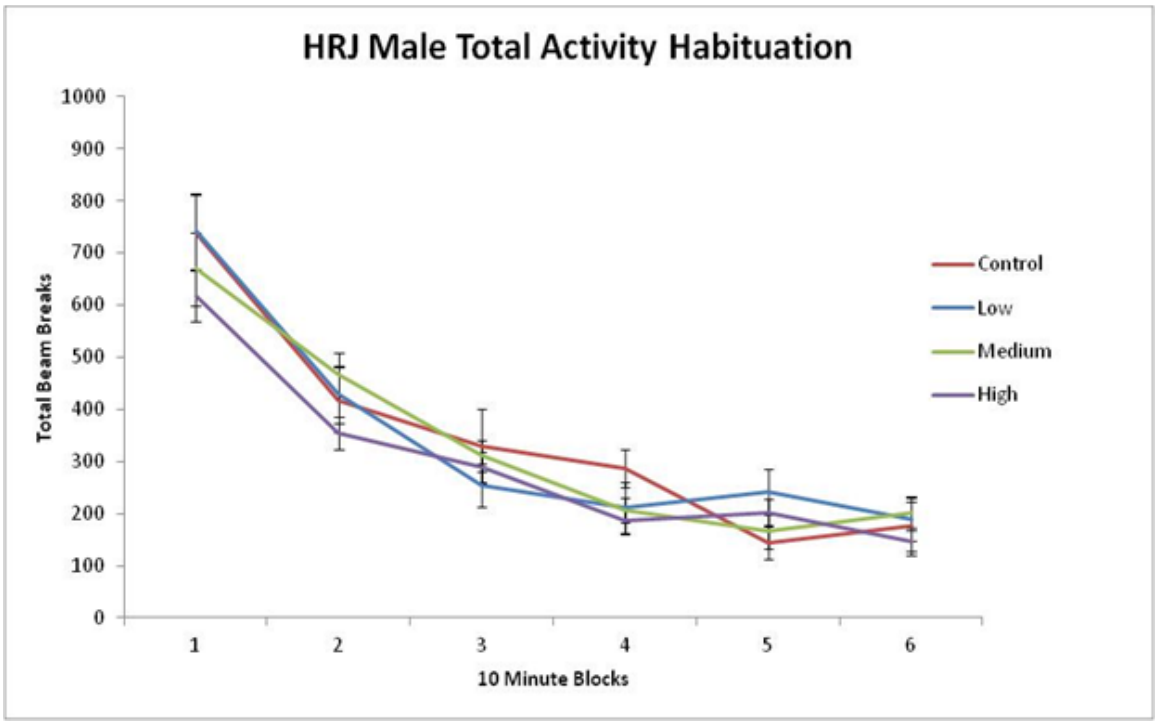

Fig 1: Motor Activity for Males

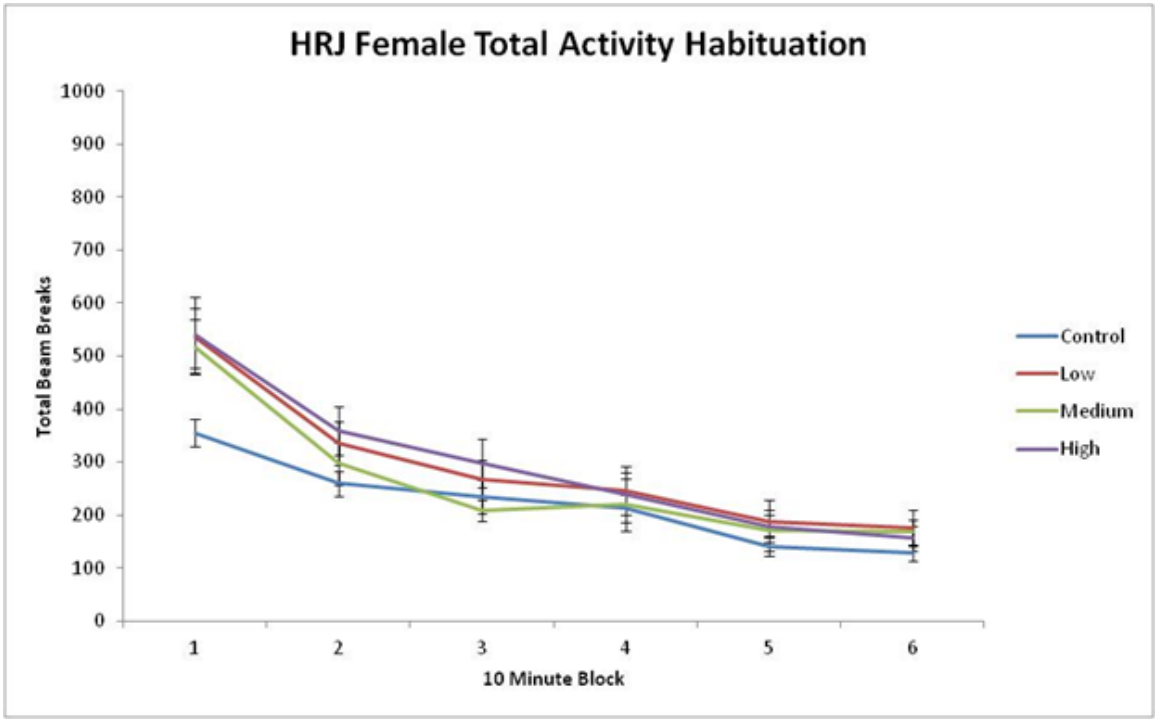

Fig 2: Motor Activity for Females

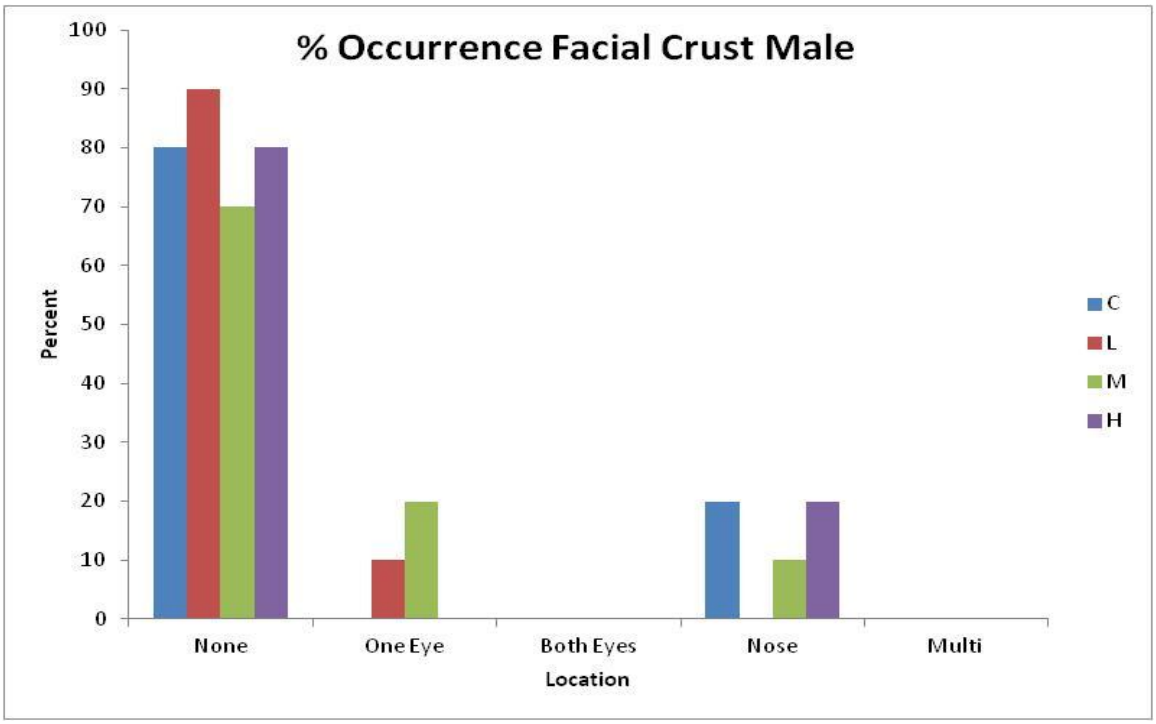

Fig 3: FOB Male Facial Crust Occurrence

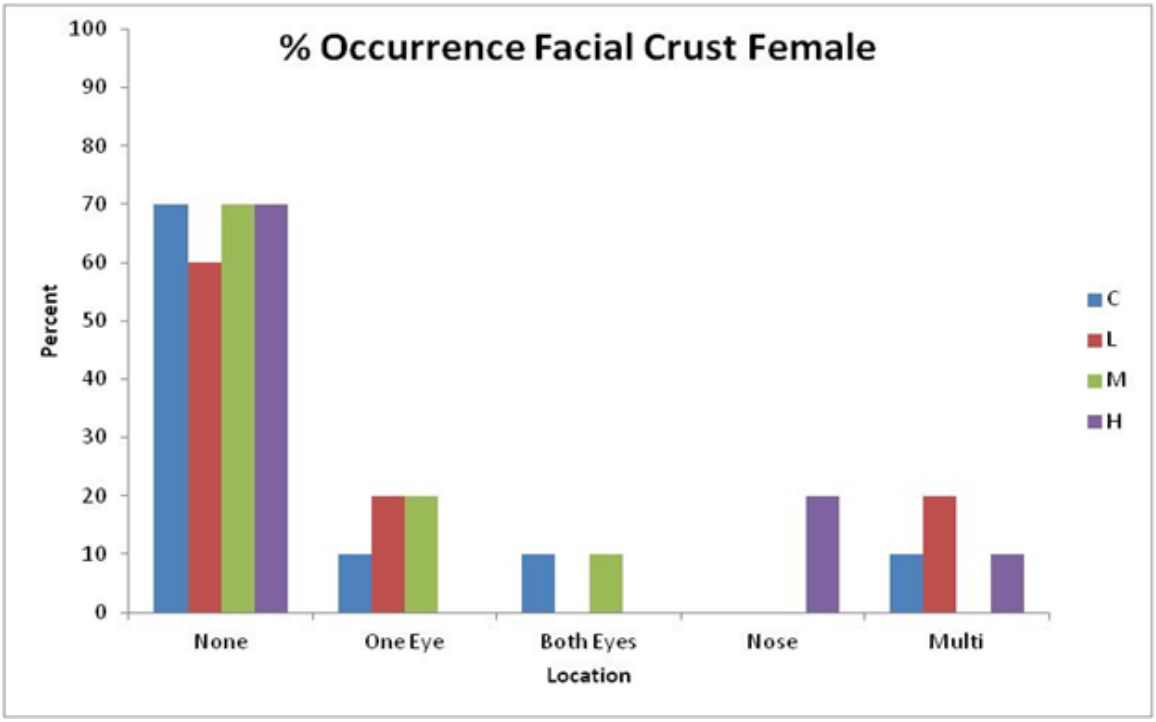

Fig 4: FOB Female Facial Crust Occurrence

APPENDIX E. SPERM MOTILITY AND CONCENTRATION IN MALE RATS EXPOSED TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

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Introduction

Exposure to some exogenous chemicals may cause malformation of sperm or reduce sperm production. A Hydrotreated Renewable Jet (HRJ) fuel produced from an extract of the camelina plant is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the US armed forces. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential toxicity of HRJ fuel by inhalation. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of FT Jet Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. In order to determine if exposure to jet fuel had any potential male reproductive effects, sperm motility and concentration were measured at the time of necropsy of male rats exposed to HRJ fuel by inhalation.

Methods

At the end of the exposure period, animals were euthanized and necropsied on August 24 and 25, 2011. For a male rat, the right testes and epididymis were immediately dissected out and individually weighed. According to the study protocol, the cauda would be removed and taken for sperm motility analysis, while the remainder of the epididymis was reweighed and frozen along with the right testes for future analysis. However, for the males necropsied on the first day and a portion on the second day, the caput (head) of the right epididymis, instead of the cauda, was taken in error, as the source for sperm for motility analysis. The cauda was taken for the last 8 males (2 per exposure group) necropsied on the second day. The left epididymis and testes were dissected and preserved in Bouin's solution for histopathology.

For sperm motility analysis, the cauda (or caput, for the initial samples) was punctured and the sperm allowed to diffuse out. A sample of the sperm was diluted using serum and an aliquot analyzed on a sperm motility analyzer (IVOS, Hamilton Thorne, Inc., Beverly, MA). Data were reported as fraction of motile sperm.

Testicular spermatid head counts and epididymal sperm counts were evaluated from the frozen right testes and frozen right epididymis (IVOS, Hamilton Thorne, Inc., Beverly, MA). The right epididymis (minus the cauda (or caput) previously removed) was thawed and reweighed prior to homogenizing for sperm count. Likewise, the right testes was thawed and reweighed for sperm count. Those samples were homogenized in buffer solution and analyzed on the sperm analyzer (IVOS, Hamilton Thorne, Inc., Beverly, MA).

Results

Motility: Sperm motility results were evaluated using several different sets of data: Motility measurements from all samples, samples from the caput only, and samples from the cauda only (Table 1 and Figure 1). The results were analyzed for differences from control. For all three cases, the differences in mean values among the exposure groups was not great enough to be statistically different from controls. The exposures were not associated with any observable differences in motility.

The data presented in Table 1 represented the final output of the sperm motility analyzer for each sample. Those data and the data analysis process of the instrument were later reviewed on the instrument computer. On the computer were found some internal sample runs that were labeled by the analysis package as having “passed” an internal measure. Those interim results were compiled (Table 2) and analyzed for statistical significance, but as with the previous results, there was no difference of the treated animals from the controls.

The staff working on the sperm analysis noted a difference in sperm quantity between the caput and cauda samples, with more coming from the cauda. Additionally, the sperm from the caput appeared to be quite fragile, with a significant and rapid decline in mobility over time. This observation is consistent with sperm physiology. Sperm are produced in the testes, and move to the caput, then through the epididymal corpus to the cauda. Sperm entering the caput are incomplete or immature, but mature as they progress to the cauda, where they are stored. Hence the observation of more fragile sperm from the caput than from the cauda is to be expected. The average motility of sperm from the caput area is lower than sperm motility from the cauda area as expected. However, comparatively, the sperm motility from the cauda area (33 to 40%) appears to be low compared with the samples that passed the motility test (Table 2), which ranged from 60 to 64%, or to a previous jet fuel study (FT jet fuel study. [reference]), where motility was in the 80-90%. This may be due to experimental differences (the FT study measured motility using microscopy and a hemacytometer) or problems with the operation of the sperm analyzer (Subsequent runs from a different experiment measured sperm motility in untreated male rats in the 80-90% range (personal communication, Mr. Shawn McInturf, 2012). Regardless of the data set, the males that were exposed to HRJ fuel did not show a difference in motility when compared with controls.

Concentration: Samples were analyzed for sperm concentration from the remainder of the right epididymis and from the right testes (Table 3). Sperm concentration measurements from the right testicle and right epididymis showed no statistically significant difference of any of the exposure groups from control (Figure 2). Exposure of male rats to HRJ fuel by inhalation did not show an effect in sperm concentration.

Table 1. HRJ Sperm Motility Summary Data

	Number	**Control**	Number	**Low**	Number	**Medium**	Number	**High**
Caput	1	0.179	21	0.000	41	0.500	61*	
	3	0.000	23	0.228	43	0.000	63	0.140
	5	0.234	25	0.021	45	0.228	65	0.091
	7	0.064	27	0.745	47	0.424	67	0.000
	9	0.630	29	0.000	49	0.459	69	0.032
	11	0.158	31	0.354	51	0.584	71	0.316
	13	0.223	33	0.235	53	0.172	73	0.209
	15	0.118	35	0.214	55	0.108	75	0.217
Cauda	17	0.511	37	0.325	57	0.486	77	0.398
	19	0.200	39	0.327	59	0.195	79	0.393
All	avg	0.232		0.245		0.316		0.199
	stdev	0.195		0.223		0.198		0.148
Caput	avg	0.201		0.225		0.309		0.143
	stdev	0.191		0.248		0.210		0.112
Cauda	avg	0.356		0.326		0.341		0.396
	stdev	0.220		0.002		0.206		0.004

*animal died prior to end of study

Table 2. HRJ Sperm Motility: Reviewed Samples that passed motility and/or rapid cells

Number	**control**	Number	**low**	Number	**medium**	Number	**high**
9	0.63	**27**	0.74	**41**	0.5	**71**	0.67
11	0.6	**31**	0.52	**51**	0.58	**77**	0.58
19	0.6			**57**	0.72	**79**	0.68
avg	0.61		0.63		0.60		0.64
stdev	0.02		0.16		0.11		0.06

Table 3. HRJ Epididymal and Testis Average Sperm Counts

	Right Epididymis		Right Testis	
Group	Average	St.Dev	Average	St.Dev
C	113.4	17.3	56.6	13.9
L	117.1	13.6	52.0	9.4
M	104.5	10.5	53.3	11.5
H	103.0	9.6	57.7	8.8

Table 4. Individual Animal Sperm Motility and Concentration

Animal #	Group	Sperm Motility (%)	Cauda Wt. (g)	Cauda Conc. (M/ml)	Epi Wt. (g)	Epi Conc. (M/ml)	Epi Sperm/ Gram	R Testes Wt. (g)	R Testes Conc. (M/ml)	R Testes Sperm/ Gram
1	C	18	0.158	1.2	0.317	127.200	381.7	1.518	62.95	188.95
3	C	0	0.222	0.3	0.301	106.15	318.5	1.526	48.85	146.5
5	C	23	0.199	1.0	0.285	98.85	296.45	1.589	54.9	164.75
7	C	6	0.175	1.2	0.272	132.400	397.3	1.106	72.5	217.5
9	C	63	0.203	2.0	0.245	121.1	363.25	1.513	51	152.9
11	C	16	0.194	3.2	0.258	96.250	288.7	1.46	40.4	121.1
13	C	22	0.250	5.1	0.300	84.450	260.9	1.837	82.35	247
15	C	12	0.229	3.8		135.5			45.85	
17	C	51	0.185	4.2	0.276	125.600	376.8	1.52	41.45	124.4
19	C	20	0.234	4.2	0.240	106.250	318.7	1.482	66.25	198.9
21	L	0	0.200	0.8	0.301	111.35	334.05	1.478	53.2	159.6
23	L	23	0.227	1.2		99.85			55.35	
25	L	2	0.200	0.6	0.279	106.3	318.9	1.403	51.75	155.25
27	L	74	0.182	0.8	0.275	123.05	369.15	1.516	54.6	163.75
29	L	0	0.188	0.8	0.2	112.05	336.1	1.323	64.2	192.65
31	L	35	0.187	4.1	0.266	121.05	359.1	1.381	58.95	176.9
33	L	23	0.215	4.1	0.325	105.8	317.5	1.608	32.3	97
35	L	21	0.201	4.1	0.25	132.65	415.15	1.511	55.35	166
37	L	33	0.218	5.0	0.273	141.5	424.55	1.5	42.45	127.45
39*	L	33	0.218	4.6	0.222	166.6	0	1.728	65.8	197.3
41	M	50	0.211	0.4	0.306	106.25	318.7	1.511	50.85	152.5
43	M	0	0.200	0.1		119.65			60.65	
45	M	23	0.211	1.1	0.298	106.6	319.35	1.459	68.9	207.8
47	M	42	0.210	1.5	0.248	104.5	313.55	1.46	55.3	165.9

Table 4. Individual Animal Sperm Motility and Concentration (continued)

		46	0.217	1.6		107.6	322.8	1.621	55.55	166.75
51	M	58	0.193	6.1	0.298	107.6	322.8	1.621	55.55	166.75
53	M	17	0.225	3.6	0.306	99.1	297.25	1.573	42.95	128.85
55	M	11	0.194	4.6	0.277	83.6	250.8	1.492	58.65	175.95
57	M	49	0.216	4.6	0.263	97.45	160.85	1.524	28.95	86.8
59	M	20	0.202	3.6	0.23	115.8	347.35	1.458	57.55	172.7
63	H	14	0.198	1.5	0.245	108.45	325.4	1.451	65.5	196.55
65	H	9	0.189	0.4	0.27	95.1	285.25	1.521	50.15	150.55
67	H	0	0.199	1.6	0.263	106.5	319.5	1.357	57.85	173.55
69	H	3	0.200	0.4	0.263	99.45	298.1	1.6	60.55	181.65
71	H	32	0.195	9.7	0.243	89.3	267.8	1.377	51.6	154.85
73	H	21	0.214	2.7	0.262	107	321.05	1.48	55.8	167.3
75	H	22	0.223	4.8		98.15			51.1	
77	H	40	0.215	4.7	0.269	100.5	324.65	1.492	50	150.05
79	H	39	0.200	3.8	0.26	122.55	367.55	1.48	76.435	226.7

* Sample removed from analysis - no volume was entered into instrument.

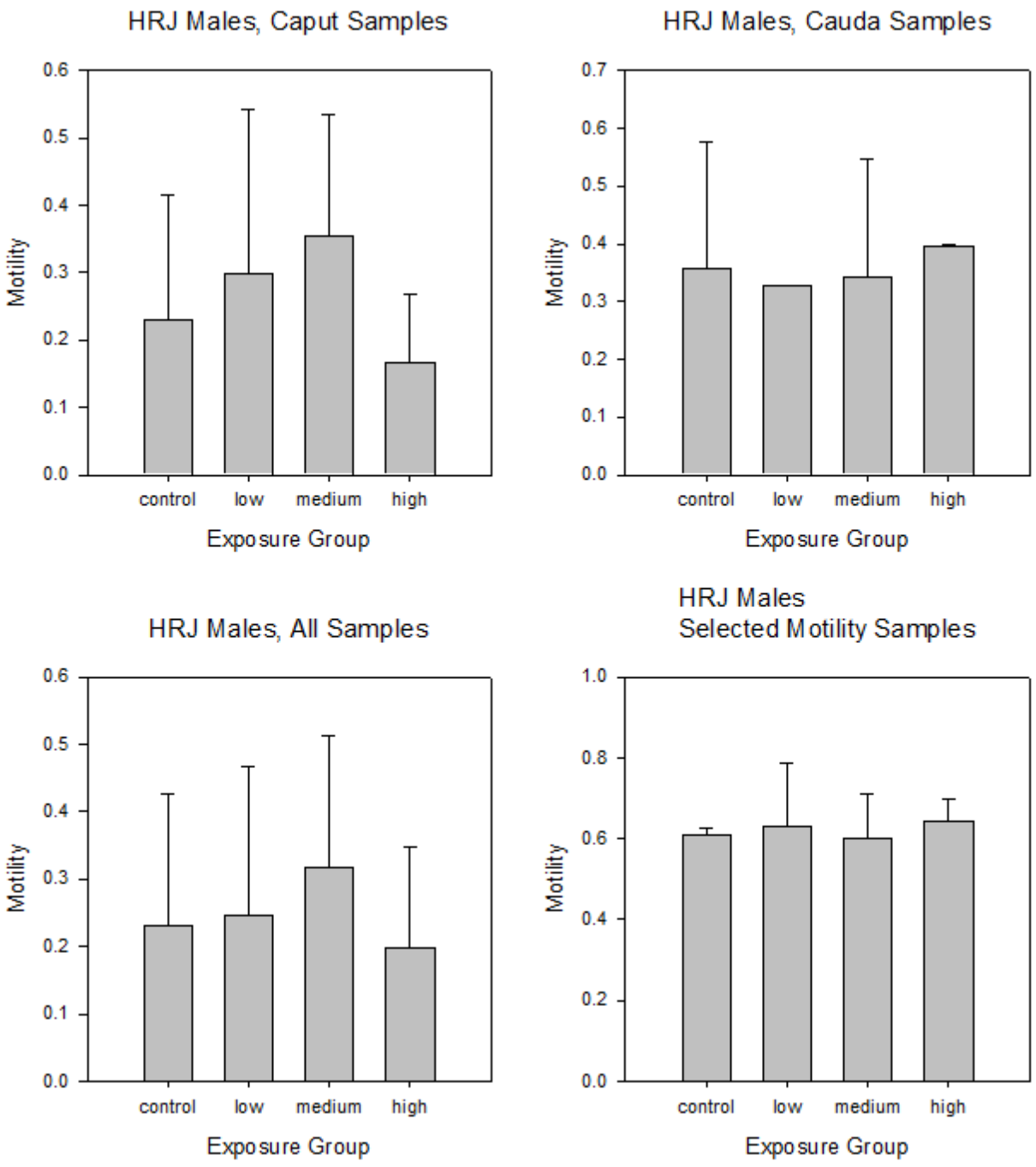

Figure 1. HRJ Average Sperm Motility Measurements

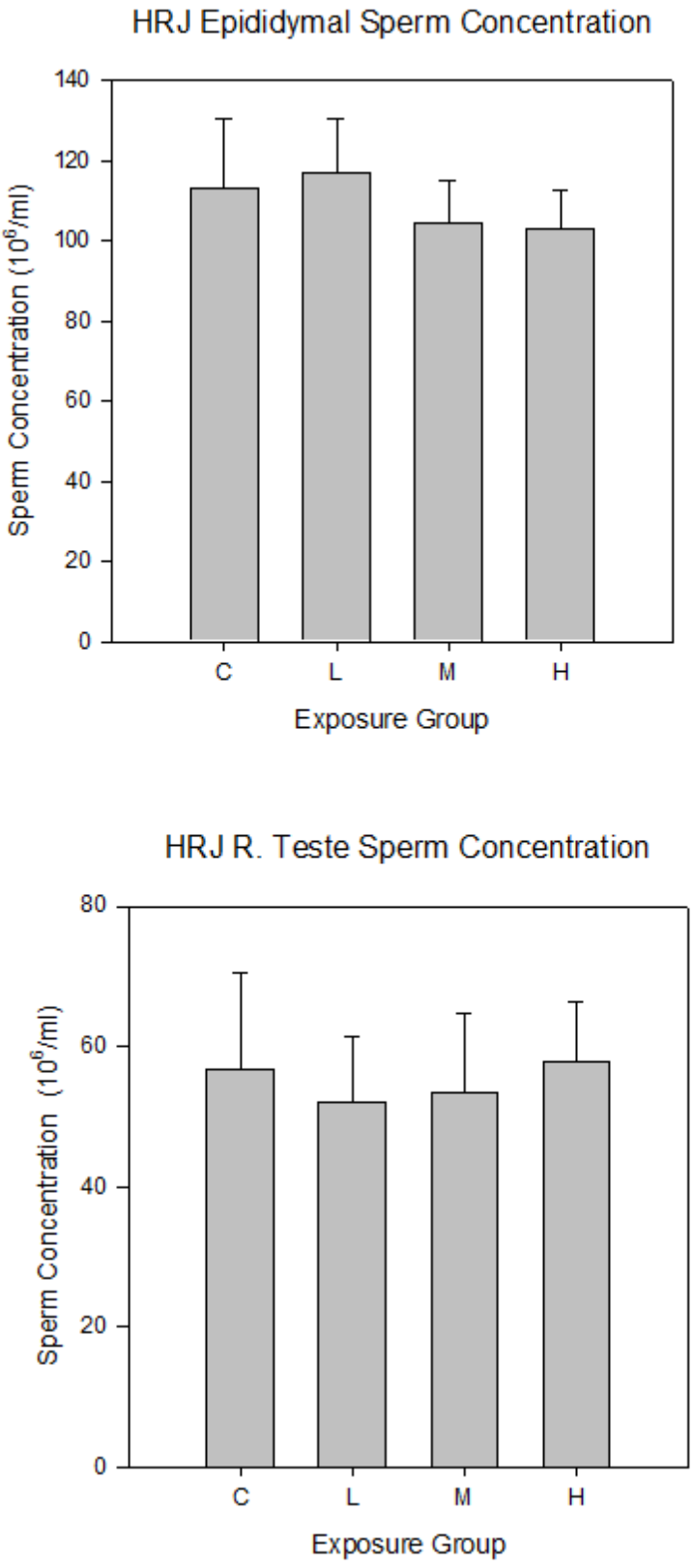

Figure 2. HRJ Average sperm Concentration Measurements

APPENDIX F. VAGINAL CYTOLOGY TO IDENTIFY ESTROUS CYCLICITY IN FEMALE RATS EXPOSED TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

Author

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Study Sponsor

U. S. Air Force, AFMC, Alternative Fuels Certification Office, ASC/WNN

REPORT PREPARATION

Report prepared by: _____ Date _____

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Introduction

Exposure to some exogenous chemicals may cause disruption of the estrous cycle in female rats (Barlow and Sullivan, 1982). A Hydrotreated Renewable Jet (HRJ) fuel produced from an extract of the camelina plant is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the US armed forces. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential toxicity of HRJ fuel by inhalation. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of FT Jet Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. In order to determine if exposure to jet fuel had any effect on the estrous cycle of the female rat, vaginal cytology was performed on each female rat over 5 day period.

Methods

Vaginal cytology was conducted on all female rats. During the 12th week of exposure, July 25 to July 29, 2011, a vaginal lavage was performed on each female rat daily, prior to exposure, over the 5 day period. Approximately 100 ul physiological saline was gently flushed into the vaginal opening and aspirated back into a pipette tip. The aspirate was placed a glass slide. The slides were prepared and read using light microscopy.

The slides were read on an optical microscope at 10 x power. The predominant vaginal cell types observed were used to identify a particular estrus stage for the day.

Results and Discussion

The vaginal lavage and cytology identified the predominant cell type present each day over a 5 day span (Table 1). The cell types identified represented the proestrus (early and late), estrus, and diestrus stages of the estrous cycle. The normal rat estrous cycle lasts from 4 to 5 days, with metestrus lasting 6-8 h, diestrus 55-57 h, proestrus 12-14 h, and estrus 25-27 h (Becker *et al.*, 2005). A female rat with a normal estrous cycle should proceed through all stages over the 5 day lavage period. In order to confirm a disruption of the estrous cycle it would be preferable to continue vaginal cytology for additional days. However, for this study, other endpoints (e.g., FOB or motor activity) were scheduled, which precluded a continuing of vaginal cytology.

The vaginal cytology for each female rat was determined over the 5 day period, and one might expect to see cytology representing all four stages. However, because some of the stages are shorter than 24 h, each stage may not be seen in the cytology (Becker *et al.*, 2005). The number of stages observed over the 5 day period for each female rat was tabulated. All of the females, showed the presence of cells representing at least two of the four stages. As vaginal cytology over additional days was not conducted, the potential effect on estrous cycle timing could not be addressed. However, all of the exposed female rats appeared to be going through the estrus cycle, regardless of exposure to the HRJ jet fuel at any concentration.

References

Barlow, S. M. and Sullivan, F. M. Reproductive Hazards of Industrial Chemicals. Academic Press, New York, 1982, p. 15.

Becker, J. B., Arnold, A. P., Berkley, K. J., Blaustein, J. D., Eckel, L. A., Hampson, E., Herman, J. P., Marts, S., Sadee, W., Steiner, M., Taylor, J., and Young, E. (2005). Strategies and Methods for Research on Sex Differences in Brain and Behavior. Endrocrinology, 146:1650-1673.

Table 1. Vaginal Cytology. Estrous Cycle Stage Identification from Slides of Vaginal Lavage

Group	Animal #	Day 1	Day 2	Day 3	Day 4	Day 5	Stages seen
Control	2	E	D	D	E	D	2
	4	D	D	D	E	D	2
	6	D	D	D	D	P	2
	66	E	E	D	E	D	2
	10	E	D	D	E	D	2
	12	E	D	D	E	D	2
	14	D	D	D	E	D	2
	16	E	E	E	D	D	2
	18	E	D	D	D	D	2
	20	D	E	E	D	D	2
Low	22	E	E	D	D	D	2
	24	P	E	P	E	P	2
	26	E	D	D	D	P	3
	28	E	E	E	D	D	2
	30	D	P	D	E	D	3
	32	D	D	P	E	P	3
	34	D	E	D	P	E	3
	36	D	E	D	E	D	2
	38	D	D	P	D	D	2
	40	D	D	D	E	E	2
Medium	42	E	E	E	D	D	2
	44	D	D	D	E	E	2
	46	E	E	D	D	D	2
	48	E	E	P	D	D	3
	50	E	D	D	D	P	3
	52	E	E	D	D	D	2
	54	D	D	D	E	E	2
	56	D	E	P	D	D	3
	58	D	D	P	E	D	3
	60	E	D	D	D	E	2
High	62	E	E	D	D	D	2
	64	E	D	D	D	D	2
	8	D	D	D	E	E	2
	68	E	E	D	D	E	2
	70	D	D	D	E	E	2
	72	D	D	E	E	D	2
	74	D	E	E	D	D	2
	76	P	E	D	D	D	2
	78	D	D	P	E	D	3
	80	D	D	E	E	E	2

M = Metestrus
D = Diestrus
P = Proestrus
E = Estrus

APPENDIX G. PATHOLOGY

AUDITED FINAL REPORT

Study Phase: Pathology

Test Site Phase Reference No. 20023782

Testing Facility Study No. F-WA-2011-0126-A

90-Day Inhalation Toxicity Study of HRJ Fuel
in Rats (Rattus norvegicus)
with Neurotoxicity Testing and Genotoxicity Assay

TESTING FACILITY:
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2729 R Street, Building 837, Area B
Wright Patterson AFB, OH 45433

TEST SITE:
Charles River Laboratories Pathology Associates, Maryland
15 Worman's Mill Court, Suite I
Frederick, Maryland 21701

COMPLIANCE STATEMENT

The portion of this study performed by Charles River Laboratories, Pathology Associates-Maryland was conducted in compliance with the protocol, amendments, and study memorandum provided by the Testing Facility Principal Investigator.

This study was conducted in accordance with the procedures described herein. The report represents an accurate and complete record of the results obtained. There were no deviations from the protocol, amendments, study memorandum or Charles River Laboratories, Pathology Associates SOPs that affected the overall integrity of the study or the interpretation of the study results and conclusions.

One protocol deviation addressing tissues missing for evaluation was sent to the Principal Investigator. Two SOP deviations were maintained with study records.

QUALITY ASSURANCE REPORT

This study has been inspected by the QAU to assure conformance with the protocol and SOPs. Reports were submitted in accordance with SOPs as follows.

		Dates Findings Submitted to:	
Dates of Inspection	Phase(s) Inspected	Study Pathologist & Test Site Management	Study Director & Management
04/04,05/2012	Individual Animal Data, Draft Pathology Report and Supporting Documentation	04/05/2012	04/13/2012
06/12/2012	Final Pathology Report	06/12/2012	06/15/2012

Enosha Simmons — 06/28/2012

Enosha Simmons
Senior Quality Assurance Auditor
Pathology Associates, Charles River Laboratories

Date

RESPONSIBLE PERSONNEL

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Histology Laboratory Manager	Fred Argilan, HTL(ASCP)SLS Charles River Laboratories, Pathology Associates, Frederick, Maryland
Research Associate	Sally Jenkins, BS Charles River Laboratories, Pathology Associates, Frederick, Maryland

SUMMARY

The objective of this study was to assess the potential inhalation toxicity of HRJ fuel, Camelina, when administered via inhalation exposure to Fischer 344 rats on a repeated basis for 90 days (5 days per week over approximately 13 weeks).

The study consisted of 4 groups (control, low, intermediate, high) of 10 male and 10 female Fischer 344 rats. HRJ fuel was administered via aerosol to control, low, intermediate and high dose groups at dose levels of 0 (control article), 200, 700, or 2000 mg/m^3, respectively for 6 hours per day, 5 days per week for approximately 13 weeks.

On the day following the last exposure, animals were euthanized, subjected to a complete gross necropsy and comprehensive tissue collection; organ weights were also measured. All tissues from animals in the control and high dose animals were processed and evaluated microscopically, along with nose, lungs, kidneys and liver from low and intermediate dose group animals.

There were two early death animals: one high dose group male and one intermediate dose female. The deaths were determined unrelated to the test material by the Test Facility and were not evaluated microscopically.

There were no HRJ fuel-related macroscopic findings observed on the necropsy day.

At the time of necropsy, HRJ fuel-related microscopic findings of goblet (mucus) cell hyperplasia and/or degeneration of the olfactory epithelium were seen in the nasal turbinates of the 2000 mg/m^3 males and females.

In conclusion, inhalation administration at three exposures plus a control (0, 200, 700, 2000 mg/m^3) to Fischer 344 rats of HRJ fuel for 5 days per week for approximately 13 weeks resulted in no test material related early deaths. At the end of the treatment period at 2000 mg/m^3 microscopic findings were noted in the nasal turbinates and included areas of goblet (mucus) cell hyperplasia and/or degeneration of the olfactory epithelium.

INTRODUCTION

This report presents the pathology findings in Fischer 344 rats assigned to the study entitled 90-Day Inhalation Toxicity Study of HRJ Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay (Study No. F-WA-2011-0126-A). The objective of this study was to assess the potential inhalation toxicity of a test substance when administered via inhalation exposure to Fischer 344 rats on a repeated basis for 90 days (5 days per week over approximately 13 weeks).

LCDR William Howard, PhD, Naval Medical Research Unit - Dayton, Wright Patterson AFB, OH, served as the Principal Investigator.

MATERIALS AND METHODS

Experimental procedures applicable to pathology investigations are summarized in Text Table 1 and Text Table 2. A protocol deviation to the pathology procedures performed by Charles River Laboratories, Pathology Associates-Maryland is listed in Appendix 1.

Text Table 1
Experimental Design

Group	Exposure Level mg/m^3	Number of Animals	
		Males	Females
Control Replicate 1	0	5	5
Control Replicate 2		5	5
Low Replicate 1	200	5	5
Low Replicate 2		5	5
Intermediate Replicate 1	700	5	5
Intermediate Replicate 2		5	5
High Replicate 1	2000	5	5
High Replicate 2		5	5
Total		40	40

All surviving animals were submitted for necropsy on the day following the last exposure. Necropsies were performed and organ weights were collected by Naval Medical Research Unit - Dayton personnel. Except as noted in Text Table 2 tissues were collected in 10% neutral buffered formalin.

Text Table 2
Tissue Collection and Examination

Provantis Tissue Term	Protocol Tissue Term	Collect	Weigh	Micro Eval	Comment
SALIVARY GLAND	Salivary glands	X	-	X	Submaxillary, sublingual, parotid.
ESOPHAGUS	Esophagus	X	-	X	-
STOMACH	Stomach	X	-	X	-
INTESTINE, DUODENUM	Duodenum	X	-	X	-
INTESTINE, JEJUNUM	Jejunum	X	-	X	-
INTESTINE, ILEUM	Ileum	X	-	X	-
INTESTINE, CECUM	Cecum	X	-	X	-
INTESTINE, COLON	Colon	X	-	X	-
INTESTINE, RECTUM	Rectum	X	-	X	-
LIVER	Liver	X	X	X	Piece from each lobe.
PANCREAS	Pancreas	X	-	X	-
BRAIN	Brain	X	X	X	Medulla /pons, cerebellum, and cerebrum.
PITUITARY GLAND	Pituitary	X	-	X	-
NERVE, SCIATIC	Peripheral sciatic nerve	X	-	X	-
SPINAL CORD, CERVICAL; SPINAL CORD, THORACIC; SPINAL CORD, LUMBAR;	Spinal cord	X	-	X	Cervical, mid-thoracic, lumbar.
EYE (LEFT)	Eyes (L)	X	-	X	-
NERVE (LEFT), OPTIC	Optic nerve, (L)	X	-	X	-
ADRENAL GLAND	Adrenal glands	X	X	X	-
THYROID GLAND (LEFT); PARATHYROID GLAND (LEFT)	Thyroid, Parathyroid (L)	X	-	X	Together.
NASAL TURBINATES-LEVEL 1; NASAL TURBINATES-LEVEL 2; NASAL TURBINATES-LEVEL 3; NASAL TURBINATES-LEVEL 4	Nose	X	-	X	4 levels.
PHARYNX	Pharynx	X	-	X	-
LARYNX	Larynx	X	-	X	-
TRACHEA	Trachea	X	-	X	With mainstem bronchi.
LUNG	Lung	X	-	X	Frontal section.
AORTA	Aorta	X	-	X	-
HEART	Heart	X	X	X	-

Provantis Tissue Term	Protocol Tissue Term	Collect	Weigh	Micro Eval	Comment
BONE MARROW, FEMUR	Bone marrow	X	-	X	-
LYMPH NODE, MESENTERIC	Lymph node, mesenteric	X	-	X	-
LYMPH NODE, BRONCHIAL	Lymph node	X	-	X	Lung associated.
SPLEEN	Spleen	X	X	X	-
THYMUS	Thymus	X	X	X	-
KIDNEY	Kidneys	X	X	X	Right and left.
URINARY BLADDER	Urinary bladder	X	-	X	-
PROSTATE GLAND	Prostate	X	-	X	-
TESTIS (LEFT)	Testes	X	X	X	Left; preserved in Bouin's solution.
EPIDIDYMIS (LEFT)	Epididymides	X	X	X	Left.
SEMINAL VESICLE (LEFT)	Seminal vesicles	X	-	X	Left with coagulatory gland.
COAGULATING GLAND (LEFT)	Coagulatory gland	X	-	X	-
UTERUS	Uterus	X	X	X	Horn, uterine body.
OVARY	Ovaries	X	X	X	-
SKIN; MAMMARY GLAND	Skin; Mammary gland	X	-	X	Skin with mammary gland-female.
LACRIMAL GLAND; HARDERIAN GLAND	Lacrimal gland; Harderian gland	X	-	X	Harderian and extra-orbital
-	Any gross lesions	X	-	X	-

Micro Eval = Microscopic Evaluation; X = procedure to be conducted; - = not required.

Tissues required for microscopic evaluation were trimmed, processed routinely, embedded in paraffin, and stained with hematoxylin and eosin (H&E) by Charles River Laboratories, Pathology Associates, Maryland. Microscopic evaluation was conducted by the undersigned board-certified veterinary pathologist on all specified tissues from all animals in Control and High dose groups and target tissues (nose, lungs, kidneys and liver) in Low and Intermediate dose groups. Tissues were evaluated by light microscopy, and the results were entered directly into a validated pathology computer program (Text Table 3) for preparation of data tables.

Computerized Systems

Critical computerized systems used in the study by the Test Site are listed below. (See Text Table 3)

Text Table 3
Computerized Systems

System Name	Version Number	Description of Data Collected and/or Analyzed
Provantis NT 2000	V3.4	Histopathology

Disposition of Study Materials

Prior to finalization of the report, pathology materials were sent to Naval Medical Research Unit - Dayton, Wright Patterson, AFB, Ohio, and the Final Report was sent to the Study Director.

RESULTS AND DISCUSSIONS

Mortality

There were two early death animals: one high dose group male and one intermediate dose female. The deaths were determined unrelated to the test material by the Test Facility and were not evaluated microscopically.

Gross Pathology

Scheduled Euthanasia Animals (End of Exposures): (Table 1, Appendix 2) No test article-related gross findings were noted.

Histopathology

Scheduled Euthanasia (End of Exposures): (Table 2, Appendix 2) Test article-related microscopic findings are summarized in Text Table 4.

Text Table 4
Summary Microscopic Findings – Scheduled Euthanasia (End of Exposures)

Group	Males				Females			
	Control	Low	Intermediate	High	Control	Low	Intermediate	High
Dose (mg/m^3)	0	200	700	2000	0	200	700	2000
No. animals examined	10	10	10	9	10	10	9	10
Nasal Turbinates – Level 1								
Hyperplasia, goblet cell								
Mild	0	0	0	7	0	0	0	2
Nasal Turbinates – Level 2								
Degeneration, olfactory epithelium								
Minimal	0	0	0	7	0	0	0	7
Mild	0	0	0	2	0	0	0	2
Hyperplasia, goblet cell								
Mild	0	0	0	8	0	0	0	10
Nasal Turbinates – Level 3								
Degeneration, olfactory epithelium								
Mild	0	0	0	9	0	0	0	10
Nasal Turbinates – Level 4*								
Degeneration, olfactory epithelium								
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	0	7	0	0	0	9

* = only 8 were evaluated for high dose males and 9 for control and high dose females.

Test article-related microscopic findings were seen in the nasal turbinates of the 2000 mg/m^3 males and females. Within the different levels of the turbinates were multifocal areas of goblet (mucus) cell hyperplasia and/or degeneration of the olfactory epithelium.

In the kidneys of male rats, chronic progressive nephropathy was seen. Minimal focal and multifocal nephropathy was observed at all exposure groups. One mild case was observed in a male rat exposed at 2000 mg/m^3. As the incidence was similar between control and exposed animals there was no exacerbation of this common background lesion in rats. Also observed were hyaline droplets in the kidney tubules of male controls and all male exposure groups. Although not recorded, the degree of droplet formation was comparable between the controls and the high dose animals.

Other microscopic findings observed, e.g., fibrosis or mononuclear infiltrate in the heart or mineralization in the trachea, were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of HRJ fuel.

CONCLUSIONS

Inhalation administration at three exposures plus a control (0, 200, 700, 2000 mg/m^3) to Fischer 344 rats of HRJ fuel for 5 days per week for approximately 13 weeks resulted in no test material

related early deaths. At the end of the treatment period at 2000 mg/m^3 microscopic findings were noted in the nasal turbinates and included areas of goblet (mucus) cell hyperplasia and/or degeneration of the olfactory epithelium.

REPORT APPROVAL

Michelle i Elliott Date: 29 Jun 2012

Michelle W. Elliott, DVM, PhD, DACVP

Study Pathologist

Charles River Laboratories, Pathology Associates, Frederick, Maryland

Table 1 Pathology - Intergroup Comparison of Gross Pathology Observations (Necropsy Day)

Removal Reason: TERMINAL EUTHANASIA	MALES					FEMALES				
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	
Number of Animals on Study :	10	10	10	9	10	10	9	10	
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)	
ADRENAL GLAND;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
AORTA;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
BONE MARROW;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
BRAIN;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
COAGULATING GLAND (LEFT);									
Submitted	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)	
No Visible Lesions	10	0	0	9	-	-	-	-	
EPIDIDYMIS (LEFT);									
Submitted	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)	
No Visible Lesions	10	0	0	9	-	-	-	-	
ESOPHAGUS;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
EYE (LEFT);									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
HEART;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9	10	0	0	10	
INTESTINE, CECUM;									
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)	

.

Removal Reason: TERMINAL EUTHANASIA	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
INTESTINE, CECUM; (continued)								
No Visible Lesions	10	0	0	9	10	0	0	10
INTESTINE, COLON;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
INTESTINE, DUODENUM;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
INTESTINE, ILEUM;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
INTESTINE, JEJUNUM;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
INTESTINE, RECTUM;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
KIDNEY;								
Submitted	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
No Visible Lesions	9	10	10	9	10	10	9	10
Dilation; left	1	0	0	0	0	0	0	0
LACRIMAL GLAND;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
LARYNX;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
LIVER;								
Submitted	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)

Removal Reason: TERMINAL EUTHANASIA	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
LIVER; (continued)								
No Visible Lesions	10	10	10	9	10	10	9	10
LUNG;								
Submitted	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
No Visible Lesions	10	10	10	9	10	10	9	10
LYMPH NODE, MESENTERIC;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
LYMPH NODE, BRONCHIAL;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
MAMMARY GLAND;								
Submitted	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(10)
No Visible Lesions	-	-	-	-	10	0	0	10
NERVE (LEFT), OPTIC;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
NERVE, SCIATIC;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
OVARY;								
Submitted	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(10)
No Visible Lesions	-	-	-	-	10	0	0	10
PANCREAS;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
PARATHYROID GLAND (LEFT);								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10

Removal Reason: TERMINAL EUTHANASIA

	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
PHARYNX;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
PITUITARY GLAND;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
PROSTATE GLAND;								
Submitted	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
No Visible Lesions	10	0	0	9	-	-	-	-
SALIVARY GLAND;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
SEMINAL VESICLE (LEFT);								
Submitted	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
No Visible Lesions	10	0	0	9	-	-	-	-
SKIN;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
SPINAL CORD, CERVICAL;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
SPINAL CORD, THORACIC;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
SPINAL CORD, LUMBAR;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
SPLEEN;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)

Removal Reason: TERMINAL EUTHANASIA

	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
SPLEEN; (continued)								
No Visible Lesions	10	0	0	9	10	0	0	10
STOMACH;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
TESTIS (LEFT);								
Submitted	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
No Visible Lesions	10	0	0	9	-	-	-	-
THYMUS;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
THYROID GLAND (LEFT);								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
TRACHEA;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
URINARY BLADDER;								
Submitted	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
No Visible Lesions	10	0	0	9	10	0	0	10
UTERUS;								
Submitted	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(10)
No Visible Lesions	-	-	-	-	10	0	0	10
NASAL TURBINATES - LEVEL 1;								
Submitted	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
No Visible Lesions	10	10	10	9	10	10	9	10
NASAL TURBINATES - LEVEL 2;								
Submitted	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
No Visible Lesions	10	10	10	9	10	10	9	10

Removal Reason: TERMINAL EUTHANASIA

	MALES					FEMALES				
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3		0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	
Number of Animals on Study :	10	10	10	9		10	10	9	10	
Number of Animals Completed:	(10)	(10)	(10)	(9)		(10)	(10)	(9)	(10)	
NASAL TURBINATES - LEVEL 3;										
Submitted	(10)	(10)	(10)	(9)		(10)	(10)	(9)	(10)	
No Visible Lesions	10	10	10	9		10	10	9	10	
NASAL TURBINATES - LEVEL 4;										
Submitted	(10)	(10)	(10)	(9)		(10)	(10)	(9)	(10)	
No Visible Lesions	10	10	10	9		10	10	9	10	
HARDERIAN GLAND;										
Submitted	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
No Visible Lesions	10	0	0	9		10	0	0	10	

Table 2 Pathology - Intergroup Comparison of Histopathology Observations (Necropsy Day)

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: TERMINAL EUTHANASIA

	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
ADRENAL GLAND;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
AORTA;								
Examined	(9)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	9	10	0	0	10
Not Examined: NOT FOUND AT TRIMMING	1	0	0	0	0	0	0	0
BONE MARROW;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
BRAIN;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
COAGULATING GLAND (LEFT);								
Examined	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
Within Normal Limits	10	0	0	9	-	-	-	-
EPIDIDYMIS (LEFT);								
Examined	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
Within Normal Limits	10	0	0	9	-	-	-	-
ESOPHAGUS;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
EYE (LEFT);								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	8	10	0	0	10
Mineralization; Cornea; focal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
HEART;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	3	0	0	1	6	0	0	7

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: TERMINAL EUTHANASIA

	MALES					FEMALES				
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3		0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	
Number of Animals on Study :	10	10	10	9		10	10	9	10	
Number of Animals Completed:	(10)	(10)	(10)	(9)		(10)	(10)	(9)	(10)	
HEART; (continued)										
Fibrosis; focal	(1)	(0)	(0)	(0)		(0)	(0)	(0)	(0)	
mild	1	0	0	0		0	0	0	0	
Fibrosis; multifocal	(0)	(0)	(0)	(2)		(0)	(0)	(0)	(0)	
minimal	0	0	0	2		0	0	0	0	
Infiltration, Mononuclear Cell; focal	(2)	(0)	(0)	(1)		(3)	(0)	(0)	(2)	
minimal	2	0	0	1		3	0	0	2	
Infiltration, Mononuclear Cell; multifocal	(4)	(0)	(0)	(6)		(1)	(0)	(0)	(1)	
minimal	4	0	0	5		1	0	0	1	
mild	0	0	0	1		0	0	0	0	
INTESTINE, CECUM;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
INTESTINE, COLON;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
INTESTINE, DUODENUM;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
INTESTINE, ILEUM;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
INTESTINE, JEJUNUM;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
INTESTINE, RECTUM;										
Examined	(10)	(0)	(0)	(9)		(10)	(0)	(0)	(10)	
Within Normal Limits	10	0	0	9		10	0	0	10	
KIDNEY;										
Examined	(10)	(10)	(10)	(9)		(10)	(10)	(9)	(10)	
Within Normal Limits	2	3	0	0		10	10	9	10	

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
KIDNEY; (continued)								
Chronic Progressive Nephropathy; focal	(0)	(1)	(2)	(0)	(0)	(0)	(0)	(0)
minimal	0	1	2	0	0	0	0	0
Chronic Progressive Nephropathy; multifocal	(8)	(6)	(8)	(9)	(0)	(0)	(0)	(0)
minimal	8	6	8	8	0	0	0	0
mild	0	0	0	1	0	0	0	0
Dilation; Pelvis	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
mild	1	0	0	0	0	0	0	0
LACRIMAL GLAND;								
Examined	(10)	(0)	(0)	(8)	(9)	(0)	(0)	(9)
Within Normal Limits	10	0	0	8	8	0	0	9
Not Examined: NOT FOUND AT TRIMMING	0	0	0	0	0	0	0	1
Not Examined: NOT PRESENT ON SLIDE	0	0	0	1	1	0	0	0
Infiltration, Lymphocytic; multifocal	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
LARYNX;								
Examined	(9)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	3	0	0	0	4	0	0	6
Not Examined: NOT FOUND AT TRIMMING	1	0	0	0	0	0	0	0
Mineralization; Submucosa; multifocal	(6)	(0)	(0)	(9)	(6)	(0)	(0)	(3)
minimal	5	0	0	9	5	0	0	3
mild	1	0	0	0	1	0	0	0
Infiltration, Mixed Cell; Submucosa; focal	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	0	0	0	0	0	0	0	1
LIVER;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
Within Normal Limits	10	10	9	9	10	10	9	10
Necrosis; hepatocellular; focal	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
minimal	0	0	1	0	0	0	0	0
LUNG;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
Within Normal Limits	7	10	9	8	9	10	9	10
Hemorrhage; focal	(1)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	1	0	0	0	1	0	0	0

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: TERMINAL EUTHANASIA

	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
LUNG; (continued)								
Hemorrhage; multifocal	(2)	(0)	(1)	(1)	(0)	(0)	(0)	(0)
minimal	2	0	1	1	0	0	0	0
LYMPH NODE, MESENTERIC;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
LYMPH NODE, BRONCHIAL;								
Examined	(7)	(0)	(0)	(8)	(5)	(0)	(0)	(7)
Within Normal Limits	6	0	0	8	5	0	0	6
Not Examined: NOT PRESENT ON SLIDE	3	0	0	1	5	0	0	3
Hemorrhage	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(1)
minimal	1	0	0	0	0	0	0	1
MAMMARY GLAND;								
Examined	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(9)
Within Normal Limits	-	-	-	-	9	0	0	9
Not Examined: NOT FOUND AT TRIMMING	-	-	-	-	0	0	0	1
Inflammation; granulomatous; focal	(-)	(-)	(-)	(-)	(1)	(0)	(0)	(0)
minimal	-	-	-	-	1	0	0	0
NASAL TURBINATES - LEVEL 1;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
Within Normal Limits	9	10	10	2	9	10	8	8
Inflammation; chronic; Submucosa; multifocal	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)
minimal	0	0	0	0	0	0	1	0
Inflammation; chronic-active; Submucosa; multifocal	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(0)
minimal	0	0	0	0	1	0	0	0
Inflammation; neutrophilic; Nasolacrimal Duct	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
Erosion; Nasolacrimal Duct; focal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
Hyperplasia; Goblet Cell; multifocal	(0)	(0)	(0)	(7)	(0)	(0)	(0)	(2)
mild	0	0	0	7	0	0	0	2
NASAL TURBINATES - LEVEL 2;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
NASAL TURBINATES - LEVEL 2; (continued)								
Within Normal Limits	8	10	9	0	7	8	8	0
Inflammation; neutrophilic; Nasolacrimal Duct	(2)	(0)	(1)	(0)	(3)	(2)	(1)	(0)
minimal	2	0	1	0	3	2	1	0
Degeneration; Olfactory Epithelium; multifocal	(0)	(0)	(0)	(9)	(0)	(0)	(0)	(9)
minimal	0	0	0	7	0	0	0	7
mild	0	0	0	2	0	0	0	2
Hyperplasia; Goblet Cell; multifocal	(0)	(0)	(0)	(8)	(0)	(0)	(0)	(10)
mild	0	0	0	8	0	0	0	10
NASAL TURBINATES - LEVEL 3;								
Examined	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
Within Normal Limits	10	10	10	0	10	10	9	0
Degeneration; Olfactory Epithelium; multifocal	(0)	(0)	(0)	(9)	(0)	(0)	(0)	(10)
mild	0	0	0	9	0	0	0	10
NASAL TURBINATES - LEVEL 4;								
Examined	(10)	(10)	(10)	(8)	(9)	(10)	(9)	(9)
Within Normal Limits	10	10	10	0	9	10	9	0
Not Examined: NOT PRESENT ON SLIDE	0	0	0	1	1	0	0	1
Degeneration; Olfactory Epithelium; multifocal	(0)	(0)	(0)	(8)	(0)	(0)	(0)	(9)
minimal	0	0	0	1	0	0	0	0
mild	0	0	0	7	0	0	0	9
NERVE (LEFT), OPTIC;								
Examined	(6)	(0)	(0)	(7)	(8)	(0)	(0)	(7)
Within Normal Limits	6	0	0	7	8	0	0	7
Not Examined: NOT PRESENT ON SLIDE	4	0	0	2	2	0	0	3
NERVE, SCIATIC;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
OVARY;								
Examined	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(10)
Within Normal Limits	-	-	-	-	10	0	0	10

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
PANCREAS;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	9	10	0	0	10
Atrophy; Acinar Cell; focal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
PARATHYROID GLAND (LEFT);								
Examined	(4)	(0)	(0)	(3)	(7)	(0)	(0)	(5)
Within Normal Limits	4	0	0	3	7	0	0	5
Not Examined: INSUFFICIENT TISSUE FOR EVALUATION	0	0	0	1	0	0	0	0
Not Examined: NOT FOUND AT TRIMMING	1	0	0	0	0	0	0	0
Not Examined: NOT PRESENT ON SLIDE	5	0	0	5	3	0	0	5
PHARYNX;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
PITUITARY GLAND;								
Examined	(6)	(0)	(0)	(7)	(7)	(0)	(0)	(10)
Within Normal Limits	6	0	0	7	7	0	0	10
Not Examined: NOT FOUND AT TRIMMING	3	0	0	2	3	0	0	0
Not Examined: NOT PRESENT ON SLIDE	1	0	0	0	0	0	0	0
PROSTATE GLAND;								
Examined	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
Within Normal Limits	7	0	0	9	-	-	-	-
Infiltration, Neutrophilic; focal	(2)	(0)	(0)	(0)	(-)	(-)	(-)	(-)
mild	2	0	0	0	-	-	-	-
Infiltration, Neutrophilic; multifocal	(1)	(0)	(0)	(0)	(-)	(-)	(-)	(-)
minimal	1	0	0	0	-	-	-	-
SALIVARY GLAND;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(9)
Within Normal Limits	10	0	0	9	10	0	0	9
Not Examined: NOT FOUND AT TRIMMING	0	0	0	0	0	0	0	1
SEMINAL VESICLE (LEFT);								
Examined	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)

Observations: Neo-Plastic and Non Neo-Plastic	MALES				FEMALES			
Removal Reason: TERMINAL EUTHANASIA	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
SEMINAL VESICLE (LEFT); (continued)								
Within Normal Limits	10	0	0	9	-	-	-	-
SKIN;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(9)
Within Normal Limits	10	0	0	9	10	0	0	9
Not Examined: NOT FOUND AT TRIMMING	0	0	0	0	0	0	0	1
SPINAL CORD, CERVICAL;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
SPINAL CORD, THORACIC;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
SPINAL CORD, LUMBAR;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
SPLEEN;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	9	10	0	0	10
Inflammation; neutrophilic; focal	(1)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
minimal	1	0	0	0	0	0	0	0
STOMACH;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
TESTIS (LEFT);								
Examined	(10)	(0)	(0)	(9)	(-)	(-)	(-)	(-)
Within Normal Limits	10	0	0	9	-	-	-	-
THYMUS;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10

Observations: Neo-Plastic and Non Neo-Plastic

Removal Reason: TERMINAL EUTHANASIA

	MALES				FEMALES			
	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
Number of Animals on Study :	10	10	10	9	10	10	9	10
Number of Animals Completed:	(10)	(10)	(10)	(9)	(10)	(10)	(9)	(10)
THYROID GLAND (LEFT);								
Examined	(9)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	9	10	0	0	10
Not Examined: NOT FOUND AT TRIMMING	1	0	0	0	0	0	0	0
TRACHEA;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	9	0	0	7	10	0	0	10
Mineralization; Submucosa; focal	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	0	0	0	1	0	0	0	0
Mineralization; Submucosa; multifocal	(1)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
minimal	1	0	0	1	0	0	0	0
URINARY BLADDER;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	10	0	0	10
UTERUS;								
Examined	(-)	(-)	(-)	(-)	(10)	(0)	(0)	(10)
Within Normal Limits	-	-	-	-	10	0	0	10
HARDERIAN GLAND;								
Examined	(10)	(0)	(0)	(9)	(10)	(0)	(0)	(10)
Within Normal Limits	10	0	0	9	6	0	0	8
Infiltration, Lymphocytic; multifocal	(0)	(0)	(0)	(0)	(4)	(0)	(0)	(2)
minimal	0	0	0	0	2	0	0	2
mild	0	0	0	0	2	0	0	0

APPENDIX 1: DEVIATIONS

DEVIATIONS

Any deviation that occurred during the portion of the study performed by Charles River Laboratories, Pathology Associates, Maryland has been authorized/acknowledged by the Principal Investigator, assessed for impact, and documented in the study records. One protocol deviation and two SOP deviations are listed below; these deviations did not impact the overall integrity of the study or the interpretation of the study results and conclusions.

Histopathology:

- Several tissues were missing for microscopic evaluation.
- Two SOP deviations were maintained with study records.

APPENDIX 2: PATHOLOGY - INDIVIDUAL ANIMAL DATA (CONCISE EDITION)

Abbreviations:
Control (Replicate 1 and 2)=Group 1
Low (Replicate 1 and 2)=Group 2
Intermediate (Replicate 1 and 2)=Group 3
High (Replicate 1 and 2)=Group 4

Animal No.: 1 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations:

	Correlated with:
KIDNEY; Dilation; left (TGL): found at trim	KIDNEY; Dilation; Pelvis; mild (H)

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART; Fibrosis; focal; mild	
KIDNEY; Chronic Progressive Nephropathy; multifocal; minimal	
Dilation; Pelvis; mild	KIDNEY; Dilation; left (G)
LARYNX; Mineralization; Submucosa; multifocal; minimal	

The following tissues were within normal limits:

ADRENAL GLAND	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)	ESOPHAGUS	
EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		NASAL TURBINATES - LEVEL 1		
NASAL TURBINATES - LEVEL 2	PANCREAS	PHARYNX	PITUITARY GLAND - LEVEL 3	NASAL TURBINATES	SALIVARY GLAND - LEVEL 4	NERVE (LEFT), OPTIC	
NERVE, SCIATIC	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	PROSTATE GLAND	SPINAL CORD, LUMBAR	SEMINAL VESICLE (LEFT)	
SKIN	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	SPLEEN	
STOMACH						HARDERIAN GLAND	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 1 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

AORTA: NOT FOUND AT TRIMMING
LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 2 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		NERVE, SCIATIC
OVARY	PANCREAS	PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)
TRACHEA	URINARY BLADDER	UTERUS	HARDERIAN GLAND			

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
NASAL TURBINATES - LEVEL 4; NOT PRESENT ON SLIDE
NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 3 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

EYE (LEFT);
Limited retina present for evaluation.

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

PITUITARY GLAND;
Pars distalis was available for evaluation

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX	PITUITARY GLAND
PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL	
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS
TRACHEA	URINARY BLADDER	HARDERIAN GLAND				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 3 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

LARYNX; NOT FOUND AT TRIMMING
PARATHYROID GLAND (LEFT); NOT FOUND AT TRIMMING
THYROID GLAND (LEFT); NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 4 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	NASAL TURBINATES - LEVEL 1	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY
PANCREAS	PHARYNX	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING
Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 5 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

PITUITARY GLAND;
Pars distalis was available for evaluation

PROSTATE GLAND;
Infiltration, Neutrophilic; focal; mild

TRACHEA;
Mineralization; Submucosa; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX	PITUITARY GLAND
SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	URINARY BLADDER
HARDERIAN GLAND						

Animal No.: 5 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 6 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LACRIMAL GLAND;
Infiltration, Lymphocytic; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

PITUITARY GLAND;
Pars distalis was available for evaluation

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL		MAMMARY GLAND
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS						

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 6 Group: 1 Sex: Female (continued)

The following tissues have not been examined:
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 7 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

PROSTATE GLAND;
Infiltration, Neutrophilic; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM
INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND	LARYNX	LIVER	LUNG
LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL		NASAL TURBINATES - LEVEL 1		
NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE, SCIATIC
PANCREAS	PARATHYROID GLAND (LEFT)		PHARYNX	SALIVARY GLAND	SEMINAL VESICLE (LEFT)	
SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN
STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 9 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

PROSTATE GLAND;
Infiltration, Neutrophilic; focal; mild

SPLEEN;
Inflammation; neutrophilic; focal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC
LYMPH NODE, BRONCHIAL		NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS
PARATHYROID GLAND (LEFT)		PHARYNX	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN
SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	STOMACH	TESTIS (LEFT)
THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 9 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 10 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3	
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)
PHARYNX	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

The following tissues have not been examined:

PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 11 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND (LEFT)	
PHARYNX	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)	SKIN		SPINAL CORD, CERVICAL
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS
THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 11 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 12 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)
PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 13 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; mild

LUNG;
Hemorrhage; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE, SCIATIC	PANCREAS	PHARYNX	PITUITARY GLAND	PROSTATE GLAND
SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA
URINARY BLADDER	HARDERIAN GLAND					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 13 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 14 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Inflammation; Submucosa; chronic-active; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

PITUITARY GLAND;
Pars distalis was available for evaluation

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC
OVARY	PANCREAS	PARATHYROID GLAND (LEFT)		PHARYNX	PITUITARY GLAND	SALIVARY GLAND
SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN
STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	UTERUS	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 15 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LACRIMAL GLAND;
One of pair was available for evaluation

LARYNX;
Mineralization; Submucosa; multifocal; minimal

LUNG;
Hemorrhage; multifocal; minimal

PANCREAS;
Atrophy; Acinar Cell; focal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE, SCIATIC	PARATHYROID GLAND (LEFT)		PHARYNX	PITUITARY GLAND

Animal No.: 15 Group: 1 Sex: Male (continued)

The following tissues were within normal limits: (continued)

PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL	
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS
THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND			

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 16 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LACRIMAL GLAND;
One of pair was available for evaluation

LARYNX;
Mineralization; Submucosa; multifocal; minimal

MAMMARY GLAND;
Inflammation; granulomatous; focal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART		
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY		
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC	LYMPH NODE, BRONCHIAL				
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC		
NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)	PHARYNX		PITUITARY GLAND		
SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL	SPINAL CORD, THORACIC	SPINAL CORD, LUMBAR				
SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	UTERUS		
HARDERIAN GLAND								

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 17 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

LYMPH NODE, BRONCHIAL;
Hemorrhage; minimal

NASAL TURBINATES - LEVEL 1;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal
Erosion; Nasolacrimal Duct; focal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

PITUITARY GLAND;
Pars distalis was available for evaluation

The following tissues were within normal limits:

ADRENAL GLAND, AORTA, BONE MARROW, BRAIN, COAGULATING GLAND (LEFT), EPIDIDYMIS (LEFT)
ESOPHAGUS, EYE (LEFT), HEART, INTESTINE, CECUM, INTESTINE, COLON, INTESTINE, DUODENUM, INTESTINE, ILEUM
INTESTINE, JEJUNUM, INTESTINE, RECTUM, LACRIMAL GLAND, LIVER, LUNG, LYMPH NODE, MESENTERIC
NASAL TURBINATES - LEVEL 3, NASAL TURBINATES - LEVEL 4, NERVE (LEFT), OPTIC, NERVE, SCIATIC, PANCREAS

Animal No.: 17 Group: 1 Sex: Male (continued)

The following tissues were within normal limits: (continued)

PHARYNX	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN
SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH
TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND	

The following tissues have not been examined:

PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 18 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Mineralization; Submucosa; multifocal; minimal

LUNG;
Hemorrhage; focal; minimal

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LIVER	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL		MAMMARY GLAND	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)
PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS						

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 18 Group: 1 Sex: Female (continued)

The following tissues have not been examined:

LACRIMAL GLAND; NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 19 Group: 1 Sex: Male Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LUNG;
Hemorrhage; focal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)	EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LARYNX	LIVER	LYMPH NODE, MESENTERIC	LYMPH NODE, BRONCHIAL
NASAL TURBINATES - LEVEL 1	PHARYNX	NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	NERVE, SCIATIC
PANCREAS	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)	SKIN
SPINAL CORD, CERVICAL	SPINAL CORD, THORACIC	SPLEEN	STOMACH
TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 19 Group: 1 Sex: Male (continued)

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 20 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	
NASAL TURBINATES - LEVEL 1		NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		
NASAL TURBINATES - LEVEL 4		NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)	
PHARYNX	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS						

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 66 Group: 1 Sex: Female Species: Rat Strain: Fischer 344

Test Material: Clean Air Dose: 0 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	NASAL TURBINATES - LEVEL 1	
NASAL TURBINATES - LEVEL 2		NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		NERVE (LEFT), OPTIC
NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)		PHARYNX	PITUITARY GLAND
SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR
SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	UTERUS
HARDERIAN GLAND						

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 21 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 22 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 23 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 24 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 25 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Canelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER LUNG NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 26 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	NASAL TURBINATES - LEVEL 2	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 27 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; focal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 28 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 29 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 30 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 31 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 32 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 33 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 34 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	NASAL TURBINATES - LEVEL 1	

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 35 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 36 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 37 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 38 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 39 Group: 2 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 40 Group: 2 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 200 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 41 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LUNG;
Hemorrhage; multifocal; minimal

The following tissues were within normal limits:

LIVER NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 42 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 43 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 44 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
One of pair was available for evaluation

NASAL TURBINATES - LEVEL 1;
Inflammation; Submucosa; chronic; multifocal; minimal

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 2 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 45 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; focal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 47 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 48 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 49 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LIVER;
Necrosis; hepatocellular; focal; minimal

The following tissues were within normal limits:

LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 50 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LIVER	LUNG	NASAL TURBINATES - LEVEL 1
NASAL TURBINATES - LEVEL 2	NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 51 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; focal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 1	
	NASAL TURBINATES - LEVEL 4	

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 52 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3 NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 53 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 54 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 55 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2	
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4		

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 56 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

KIDNEY LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 57 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

The following tissues were within normal limits:

LIVER	LUNG	NASAL TURBINATES - LEVEL 1	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3		NASAL TURBINATES - LEVEL 4	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 58 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LUNG	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 59 Group: 3 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Inflammation; Nasolacrimal Duct; neutrophilic; minimal

The following tissues were within normal limits:

LIVER LUNG NASAL TURBINATES - LEVEL 1 NASAL TURBINATES - LEVEL 3
NASAL TURBINATES - LEVEL 4

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 60 Group: 3 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 700 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations: None

The following tissues were within normal limits:

KIDNEY	LUNG	NASAL TURBINATES - LEVEL 2
NASAL TURBINATES - LEVEL 3	NASAL TURBINATES - LEVEL 4	
LIVER	NASAL TURBINATES - LEVEL 1	

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 8 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LACRIMAL GLAND;
One of pair was available for evaluation

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

OVARY;
One of pair was available for evaluation

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		NERVE (LEFT), OPTIC
NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SPINAL CORD, CERVICAL
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)

Animal No.: 8 Group: 4 Sex: Female (continued)

The following tissues were within normal limits: (continued)

TRACHEA URINARY BLADDER UTERUS HARDERIAN GLAND

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
MAMMARY GLAND; NOT FOUND AT TRIMMING
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE
SKIN; NOT FOUND AT TRIMMING

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 62 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

ADRENAL GLAND;
Cortex and one medulla were available for evaluation

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)
PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

Animal No.: 62 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 63 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; mild
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND AORTA BONE MARROW BRAIN COAGULATING GLAND (LEFT) EPIDIDYMIS (LEFT)
ESOPHAGUS EYE (LEFT) INTESTINE, CECUM INTESTINE, COLON INTESTINE, DUODENUM INTESTINE, ILEUM INTESTINE, JEJUNUM
INTESTINE, RECTUM LACRIMAL GLAND LIVER LUNG LYMPH NODE, MESENTERIC LYMPH NODE, BRONCHIAL
NASAL TURBINATES - LEVEL 1 NERVE (LEFT), OPTIC NERVE, SCIATIC PANCREAS PARATHYROID GLAND (LEFT)

Animal No.: 63 Group: 4 Sex: Male (continued)

The following tissues were within normal limits: (continued)

PHARYNX	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN
SPINAL CORD, CERVICAL	THYMUS	SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH
TESTIS (LEFT)		THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND	

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 64 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART	
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	
LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	
PARATHYROID GLAND (LEFT)	PHARYNX		PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL	
SPINAL CORD, THORACIC	SPINAL CORD, LUMBAR		SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	
TRACHEA	URINARY BLADDER	UTERUS	HARDERIAN GLAND				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 65 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; mild
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 65 Group: 4 Sex: Male (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)	INTESTINE, ILEUM	EPIDIDYMIS (LEFT)	
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	LYMPH NODE, BRONCHIAL	INTESTINE, JEJUNUM	
INTESTINE, RECTUM	LIVER	LUNG	LYMPH NODE, MESENTERIC	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	
NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX	SPINAL CORD, CERVICAL	SPINAL CORD, THORACIC		
SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, LUMBAR	THYMUS	THYROID GLAND (LEFT)	TRACHEA	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)				
URINARY BLADDER	HARDERIAN GLAND						

The following tissues have not been examined:

LACRIMAL GLAND; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 67 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LACRIMAL GLAND;
One of pair was available for evaluation

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 67 Group: 4 Sex: Male (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)			EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM		INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC			LYMPH NODE, BRONCHIAL
NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX		PITUITARY GLAND
PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL		
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)		THYMUS
THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND				

The following tissues have not been examined:

PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 68 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	
NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 68 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 69 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

EYE (LEFT);
Mineralization; Cornea; focal; minimal: Mutinucleated giant cell present.

HEART;
Infiltration, Mononuclear Cell; multifocal; mild

KIDNEY;
Chronic Progressive Nephropathy; multifocal; mild

LACRIMAL GLAND;
One of pair was available for evaluation

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild
Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 69 Group: 4 Sex: Male (continued)

Histo Pathology Observations: (continued)

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

PITUITARY GLAND;
Pars distalis was available for evaluation

TRACHEA;
Mineralization; Submucosa; focal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)	EPIDIDYMIS (LEFT)	
ESOPHAGUS	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
NERVE, SCIATIC	PANCREAS	PHARYNX	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)
SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN
STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	URINARY BLADDER	HARDERIAN GLAND	

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); INSUFFICIENT TISSUE FOR EVALUATION

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 70 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 24AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 24AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; focal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

PITUITARY GLAND;
Pars distalis was available for evaluation

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM
INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY	LACRIMAL GLAND
LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)

Animal No.: 70 Group: 4 Sex: Female (continued)

The following tissues were within normal limits: (continued)

PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS						

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 71 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 71 Group: 4 Sex: Male (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL
NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)
SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN
STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND

The following tissues have not been examined:

PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 72 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

PITUITARY GLAND;
Pars distalis was available for evaluation

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS
PARATHYROID GLAND (LEFT)		PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)
TRACHEA	URINARY BLADDER	UTERUS	HARDERIAN GLAND			

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 72 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

NASAL TURBINATES - LEVEL 4; NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 73 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)			EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	HEART	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	
INTESTINE, JEJUNUM	INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		
LYMPH NODE, BRONCHIAL	PITUITARY GLAND	NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND (LEFT)		
PHARYNX	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)	SKIN			
SPINAL CORD, CERVICAL	SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH		
TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND		

Animal No.: 73 Group: 4 Sex: Male (continued)

The following tissues have not been examined:

NASAL TURBINATES - LEVEL 4; NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 74 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; mild
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

HARDERIAN GLAND;
Infiltration, Lymphocytic; multifocal; minimal

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND	NERVE (LEFT), OPTIC
NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX	PITUITARY GLAND	SALIVARY GLAND	SKIN

Animal No.: 74 Group: 4 Sex: Female (continued)

The following tissues were within normal limits: (continued)

SPINAL CORD, CERVICAL SPINAL CORD, THORACIC SPINAL CORD, LUMBAR SPLEEN STOMACH
THYMUS THYROID GLAND (LEFT) TRACHEA URINARY BLADDER UTERUS

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used: TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 75 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Fibrosis; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

TRACHEA;
Mineralization; Submucosa; multifocal; minimal
Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 75 Group: 4 Sex: Male (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL
NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PARATHYROID GLAND (LEFT)	PHARYNX		PITUITARY GLAND
PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)	SKIN	SPINAL CORD, CERVICAL		
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS
THYROID GLAND (LEFT)	URINARY BLADDER	HARDERIAN GLAND				

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 76 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Infiltration, Mixed Cell; Submucosa; focal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

PITUITARY GLAND;
Pars distalis was available for evaluation

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL		MAMMARY GLAND
NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PARATHYROID GLAND (LEFT)
PHARYNX	PITUITARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

Animal No.: 76 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

LACRIMAL GLAND; NOT FOUND AT TRIMMING
SALIVARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 77 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

ADRENAL GLAND;
One of pair was available for evaluation

HEART;
Fibrosis; multifocal; minimal
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 77 Group: 4 Sex: Male (continued)

Histo Pathology Observations: (continued)

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL
NERVE (LEFT), OPTIC	NERVE, SCIATIC	PANCREAS	PHARYNX	PITUITARY GLAND	PROSTATE GLAND	SALIVARY GLAND
SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS	THYROID GLAND (LEFT)	TRACHEA
URINARY BLADDER	HARDERIAN GLAND					

The following tissues have not been examined:

PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 78 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LYMPH NODE, BRONCHIAL;
Hemorrhage; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LARYNX	LIVER	LUNG	LYMPH NODE, MESENTERIC		MAMMARY GLAND
NASAL TURBINATES - LEVEL 1		NERVE (LEFT), OPTIC	NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 78 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 79 Group: 4 Sex: Male Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

HEART;
Infiltration, Mononuclear Cell; multifocal; minimal

KIDNEY;
Chronic Progressive Nephropathy; multifocal; minimal

LARYNX;
Mineralization; Submucosa; multifocal; minimal

LUNG;
Hemorrhage; multifocal; minimal

NASAL TURBINATES - LEVEL 1;
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; minimal
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; minimal
Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 79 Group: 4 Sex: Male (continued)

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	COAGULATING GLAND (LEFT)		EPIDIDYMIS (LEFT)
ESOPHAGUS	EYE (LEFT)	INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM
INTESTINE, RECTUM	LACRIMAL GLAND	LIVER	LYMPH NODE, MESENTERIC		NERVE, SCIATIC	PANCREAS
PHARYNX	PROSTATE GLAND	SALIVARY GLAND	SEMINAL VESICLE (LEFT)		SKIN	SPINAL CORD, CERVICAL
SPINAL CORD, THORACIC		SPINAL CORD, LUMBAR	SPLEEN	STOMACH	TESTIS (LEFT)	THYMUS
THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER	HARDERIAN GLAND			

The following tissues have not been examined:

LYMPH NODE, BRONCHIAL; NOT PRESENT ON SLIDE
NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE
PITUITARY GLAND; NOT FOUND AT TRIMMING

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 80 Group: 4 Sex: Female Species: Rat Strain: Fischer 344

Test Material: HRJ Camelina Dose: 2000 mg/m^3 Route: Inhalation Study Type: Inhalation
Date of Death : 25AUG2011 Study Day No. (Week): 90 (13) Mode of Death: TERMINAL EUTHANASIA
Date of Necropsy: 25AUG2011 ** NECROPSY COMPLETE **

Pathologist: Michelle W. Elliott ** EXAMINATION COMPLETE **

Terminal Body Weight: None

Gross Pathology Observations: None

Any remaining protocol required tissues, which have been examined, have no visible lesions

Histo Pathology Observations:

LARYNX;
Mineralization; Submucosa; multifocal; minimal

NASAL TURBINATES - LEVEL 2;
Degeneration; Olfactory Epithelium; multifocal; mild
Hyperplasia; Goblet Cell; multifocal; mild

NASAL TURBINATES - LEVEL 3;
Degeneration; Olfactory Epithelium; multifocal; mild

NASAL TURBINATES - LEVEL 4;
Degeneration; Olfactory Epithelium; multifocal; mild

The following tissues were within normal limits:

ADRENAL GLAND	AORTA	BONE MARROW	BRAIN	ESOPHAGUS	EYE (LEFT)	HEART
INTESTINE, CECUM	INTESTINE, COLON	INTESTINE, DUODENUM	INTESTINE, ILEUM	INTESTINE, JEJUNUM	INTESTINE, RECTUM	KIDNEY
LACRIMAL GLAND	LIVER	LUNG	LYMPH NODE, MESENTERIC		LYMPH NODE, BRONCHIAL	
MAMMARY GLAND	NASAL TURBINATES - LEVEL 1		NERVE, SCIATIC	OVARY	PANCREAS	PHARYNX
PITUITARY GLAND	SALIVARY GLAND	SKIN	SPINAL CORD, CERVICAL		SPINAL CORD, THORACIC	
SPINAL CORD, LUMBAR	SPLEEN	STOMACH	THYMUS	THYROID GLAND (LEFT)	TRACHEA	URINARY BLADDER
UTERUS	HARDERIAN GLAND					

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

Animal No.: 80 Group: 4 Sex: Female (continued)

The following tissues have not been examined:

NERVE (LEFT), OPTIC; NOT PRESENT ON SLIDE
PARATHYROID GLAND (LEFT); NOT PRESENT ON SLIDE

Codes Used:TGL = Trackable Gross Lesion, G = Gross Finding, H = Histo Finding.

APPENDIX H. CLINICAL CHEMISTRY AND HEMATOLOGY MEASUREMENTS IN RATS EXPOSED TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

Author

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Study Sponsor

U. S. Air Force, AFMC, Alternative Fuels Certification Office, ASC/WNN

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Introduction

Male and Female Fisher F344 rats were exposed to an aerosol and vapor mixture of Hydrotreated Renewable Jet (HRJ) fuel by whole-body inhalation. Rats were exposed 6 hr/day, 5 d/wk over an approximately 90 days. At the end of the exposure period, all animals were euthanized and necropsied.

Methods

At the terminal necropsy, blood samples were taken for hematology and clinical chemistry. Samples of whole blood with anticoagulant were analyzed using a blood analyzer (Hemavet 950, Drew Scientific, Dallas, TX), while samples of plasma were analyzed using a chemistry analyzer (Vet Test 8008 and Vet Lyte, IDEXX Laboratories, Westbrook, ME). A sample of whole blood was analyzed for blood clotting time. Prothrombin Time (PT) test and international normalized ratio (INR) were determined using a blood clot analyzer (GEM PCL Plus from Instrumentation Laboratory, Lexington, MA).

The blood samples were analyzed for leukocyte count (WBC), leukocyte differentials (neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO), basophils (BA), erythrocyte count (RBC), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT), red blood cell distribution width (RDW), and mean platelet volume (MPV).

Plasma samples were analyzed for albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), cholesterol (CHOL), creatine kinase (CK) creatinine (CREA), Globulin (GLOB), glucose (GLU), total bilirubin (TBIL), total protein (TP), triglycerides (TRIG), and the ions sodium (NA+), potassium (K+), and chloride (CL-).

Data Analysis

Data were tabulated and analyzed for significance (SigmaPlot, Systat Software, Inc., Chicago, IL). A one-way analysis of variance (ANOVA) was conducted after a test for normality and equality of variance. If the ANOVA was significant ($P < 0.05$), groups were compared using the Holm-Sidak test. If the test for normality or equality of variance failed, a Kruskal Wallis test was performed. If that test was significant, Dunn's test was used to compare significance among groups.

Results

Clotting effectiveness as expressed by the prothrombin time did not significantly differ for either male or female rats in any of the exposure groups compared with the control animals. There

were no significant differences in hematological parameters (WBC, RBC, etc.) between control and exposed animals at any concentration for male or female rats.

For male rats, there was an elevated albumin and total protein in the low concentration animals (3.64 mg/ml) as compared with controls (3.18), but not the higher concentration animals. In addition, the total bilirubin was also elevated in the male rats exposed at the lower concentration, but not at the intermediate or high concentration groups. None of the other clinical chemistry parameters were significantly different in the exposed animals compared with controls.

While decreased levels of total protein or albumin can be associated with liver dysfunction, reasons for elevated levels of albumin and total protein found in this study are not apparent. Since albumin constitutes a significant portion of total protein, the elevated total protein results from the elevated albumin, a correlation which can be seen in the individual animal data (data not shown). Increased albumin is rare, but may be associated with dehydration. Other factors associated with dehydration such as increased concentrations of electrolytes (Na+, K+ or Cl-) are not seen. Liver enzymes (AST, ALP, and ALT) were not significantly different from controls, so there is no other evidence for liver injury. Increased total bilirubin may be a sign of increased hemolysis or the incapacity of the liver to remove bilirubin, but there is no other evidence for liver damage as the liver enzymes in the exposed groups are not significantly different from the control group.

In female rats, the only clinical chemistry parameter that was significantly different was globulin, which was slightly decreased in rats exposed at the low concentration, only. Upon inspection of the individual animal data, there was one animal, (no. 32) in which the globulin value was much lower (1.2 g/dL) compared with the other animals in the group (between 2.1 and 2.3 g/dL). Also in that animal, albumin was elevated (4.5 vs. 2.2 to 3.3 g/dL for the other females in the group). An increased albumin to globulin ratio may be related to an immune disorder characterized by a reduction in globulins. As only one animal exhibited the decreased globulin and increased albumin, with no other clinical chemistry effects, and no dose response with the other exposure groups, the biological significance of the response not considered to be related to the animal exposure to the test material.

Table 1. Clinical Chemistry Results, Males

Group		ALB	ALKP	ALT	AST	BUN	CHOL	CK	CREA	GLOB	GLU	TBIL	TP	TRIG	Na+	K+	Cl-
Control	Mean	3.2	100	61	78	19	50	730	0.4	2.7	193	0.2	5.9	105	148	4.5	105
	SD	0.2	28	11	11	3	8	396	0.1	0.1	17	0.1	0.3	63	1	0.3	1
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Low	Mean	3.6	121	70	95	21	52	870	0.5	3.0	202	0.4	6.6	115	147	4.8	103
	SD	0.3	28	12	18	4	8	492	0.1	0.3	78	0.1	0.6	60	7	0.5	4
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mid	Mean	3.1	97	62	92	18	44	650	0.4	2.7	206	0.3	5.8	98	148	5.1	105
	SD	0.2	18	29	34	2	6	327	0.1	0.1	16	0.1	0.2	58	2	0.5	1
	N	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10
High	Mean	3.1	90	57	77	19	44	404	0.5	2.7	195	0.2	5.8	65	147	4.8	105
	SD	0.2	15	12	32	3	7	261	0.1	0.1	15	0.2	0.3	35	1	0.4	1
	N	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10

Table 2. Clinical Chemistry Results, Females

Group		ALB	ALKP	ALT	AST	BUN	CHOL	CK	CREA	GLOB	GLU	TBIL	TP	TRIG	Na+	K+	Cl-
Control	Mean	3.1	83	60	79	19	70	615	0.4	2.5	182	0.2	5.5	35	148	4.7	107
	SD	0.1	21	10	12	2	6	358	0.05	0.2	21	0.1	0.2	13	1	0.4	1
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Low	Mean	3.2	82	55	69	17	70	402	0.4	2.1	171	0.1	5.3	32	148	4.9	107
	SD	0.6	26	8	9	3	7	273	0.03	0.3	23	0.05	0.4	7	1	0.3	1
	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Mid	Mean	3.1	69	57	75	17	71	549	0.3	2.3	165	0.2	5.4	38	148	4.7	107
	SD	0.2	7	13	13	2	11	322	0.05	0.2	24	0.1	0.2	7	1	0.2	1
	N	9	8	9	9	9	9	9	9	9	9	9	9	9	9	9	9
High	Mean	3.1	70	55	78	20	72	512	0.4	2.3	161	0.3	5.4	30	147	5.1	107
	SD	0.2	37	12	19	2	5	359	0.05	0.1	18	0.3	0.3	9	1	0.5	1
	N	10	8	10	10	10	10	9	10	10	10	10	10	10	10	10	10

Table 3. Hematology Results, Males

Group		WBC	NE#	LY#	MO#	EO#	BA#	NE%	LY%	MO%	EO%	BA%
Control	Mean	5.35	1.70	3.00	0.63	0.03	0.00	31.88	55.66	11.92	0.45	0.09
	SD	0.97	0.31	0.67	0.22	0.02	0.01	3.58	4.09	4.01	0.34	0.12
	N	10	10	10	10	10	10	10	10	10	10	10
Low	Mean	5.21	1.75	2.75	0.68	0.02	0.01	33.63	52.74	12.95	0.42	0.25
	SD	0.86	0.33	0.61	0.30	0.02	0.02	4.30	8.40	5.08	0.43	0.44
	N	10	10	10	10	10	10	10	10	10	10	10
Mid	Mean	5.10	1.56	2.80	0.72	0.03	0.00	30.73	54.73	14.00	0.51	0.03
	SD	1.14	0.41	0.79	0.31	0.02	0.00	5.10	8.09	4.12	0.44	0.06
	N	10	10	10	10	10	10	10	10	10	10	10
High	Mean	5.94	1.84	3.27	0.76	0.04	0.02	31.53	54.72	12.81	0.65	0.29
	SD	1.36	0.29	0.92	0.24	0.06	0.04	3.47	4.44	3.24	0.82	0.54
	N	9	9	9	9	9	9	9	9	9	9	9

Table 3. Hematology Results, Males (continued)

Group		RBC	HB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	PDW
Control	Mean	9.2	16.3	53.8	58.5	17.8	30.4	16.1	807	7.3	0
	SD	0.6	0.9	2.8	1.2	0.8	0.8	0.7	211	1.2	0
	N	10	10	10	10	10	10	10	10	10	10
Low	Mean	9.2	16.6	53.9	58.8	18.1	30.8	16.3	862	7.1	0
	SD	0.6	1.2	3.9	0.8	0.3	0.6	0.7	200	0.3	0
	N	10	10	10	10	10	10	10	10	10	10
Mid	Mean	8.9	16.1	52.8	59.2	18.0	30.5	15.8	833	7.0	0
	SD	0.6	1.2	2.6	2.5	0.8	1.1	0.7	121	0.4	0
	N	10	10	10	10	10	10	10	10	10	10
High	Mean	9.3	16.6	54.3	58.5	17.9	30.6	16.0	766	7.1	0
	SD	0.4	1.0	3.2	0.8	0.4	0.6	0.4	197	0.6	0
	N	9	9	9	9	9	9	9	9	9	9

Table 4. Hematology Results, Females

Group		WBC	NE#	LY#	MO#	EO#	BA#	NE%	LY%	MO%	EO%	BA%
Control	Mean	4.10	1.08	2.41	0.60	0.01	0.00	26.43	58.62	14.69	0.25	0.02
	SD	0.63	0.26	0.45	0.26	0.01	0.00	4.19	5.86	6.47	0.24	0.05
	N	10	10	10	10	10	10	10	10	10	10	10
Low	Mean	4.36	1.10	2.56	0.70	0.02	0.00	25.03	58.85	15.70	0.40	0.01
	SD	0.80	0.31	0.47	0.33	0.02	0.00	4.75	5.78	6.34	0.48	0.04
	N	10	10	10	10	10	10	10	10	10	10	10
Mid	Mean	4.13	0.96	2.52	0.64	0.01	0.00	23.56	61.20	15.01	0.18	0.04
	SD	0.74	0.21	0.45	0.26	0.01	0.00	4.31	5.77	4.16	0.21	0.12
	N	9	9	9	9	9	9	9	9	9	9	9
High	Mean	3.91	0.87	2.44	0.59	0.01	0.00	22.16	63.13	14.32	0.34	0.06
	SD	0.89	0.22	0.45	0.41	0.02	0.01	2.20	7.05	6.74	0.40	0.15
	N	10	10	10	10	10	10	10	10	10	10	10

Table 4. Hematology Results, Females (continued)

Group		RBC	HB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	PDW
Control	Mean	8.2	15.9	52.8	64.6	19.4	30.1	14.4	879	6.8	0
	SD	0.4	0.8	2.6	0.6	0.6	0.9	0.4	69	0.4	0
	N	10	10	10	10	10	10	10	10	10	10
Low	Mean	8.2	16.1	52.9	64.4	19.6	30.4	14.8	944	6.7	0
	SD	0.4	0.8	2.3	0.7	0.6	0.8	0.7	88	0.4	0
	N	10	10	10	10	10	10	10	10	10	10
Mid	Mean	7.8	15.3	50.9	64.8	19.5	30.1	14.4	830	6.8	0
	SD	0.6	1.3	3.7	0.5	0.7	1.1	0.4	116	0.3	0
	N	9	9	9	9	9	9	9	9	9	9
High	Mean	8.3	15.9	53.3	64.0	19.1	29.8	14.3	796	6.9	0
	SD	0.4	0.6	2.3	0.4	0.5	0.7	0.3	201	0.7	0
	N	10	10	10	10	10	10	10	10	10	10

APPENDIX I. MEASUREMENT OF THE PROTEIN ALPHA 2-MICROGLOBULIN IN KIDNEY SAMPLES FROM F344 RATS EXPOSED BY INHALATION TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

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INTRODUCTION

The compound alpha 2-microglobulin is a low molecular weight protein that is synthesized in the male rat liver. Synthesis of alpha 2-microglobulin is initiated in the male rat with the onset of sexual maturation, increases in the young adult, plateaus around 20 weeks of age, and then decreases with aging. The protein is secreted into the plasma and is filtered through the kidney. About half of the alpha 2-microglobulin is excreted in the urine, and half is reabsorbed into the proximal tubule of the kidney nephron, where it undergoes hydrolytic digestion. The female rat liver does not produce alpha 2-microglobulin and levels of the protein in the urine are approximately two orders of magnitude lower than in the rat (Swenberg, 1993).

Exposure to certain chemicals (especially branched hydrocarbons) is associated with the accumulation of alpha 2-microglobulin in renal tubular epithelial cells. These chemicals have been hypothesized to form a complex with alpha 2-microglobulin that resists lysosomal degradation, leading to accumulation in protein (hyaline) droplets in the cells. The accumulation of alpha 2-microglobulin leads to cytotoxicity where injured cells are released into the tubule lumen, and leaving casts in the loop of Henle, or being excreted into the urine. The cytotoxicity also causes regeneration of cells in the area (Borghoff *et al.*, 1990). Histopathologically, alpha 2-microglobulin nephropathy is characterized by an excessive accumulation of protein droplet in lysosomes of the proximal tubules, the presence of cellular casts from injured cells in the junction of the proximal tubule and the loop of Henle, and regenerative tubules (Borghoff *et al.*, 1990). Various chemicals have been shown to cause alpha 2-microglobulin nephropathy, including some jet fuel formulations (Swenberg, 1993).

In long-term toxicology studies of some hydrocarbon chemicals, some renal tumors have been observed to occur in male rats only. In contrast to other renal tumors that occur in both males and females, research has linked the origin of those male rat kidney tumors to the accumulation of alpha 2-microglobulin and subsequent nephropathy. As female rats and other laboratory species do not demonstrate the renal lesions related to alpha 2-microglobulin accumulation, the U.S. EPA has advised that male rats are not a good model for assessing human risk from exposure to compounds that induce alpha 2-microglobulin accumulation (U.S. EPA, 1991).

A Hydrotreated Renewable Jet (HRJ) fuel produced from an extract of the camelina plant is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the US armed forces. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential toxicity of HRJ fuel by inhalation. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of HRJ fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females. As the HRJ fuel is composed of hydrocarbons, alpha 2-microglobulin was measured in kidney samples to provide data for assessing whether or not HRJ fuel should be considered to be a chemical that induces alpha 2-microglobulin accumulation.

METHODS

Alpha 2-Microglobulin Purification and ELISA

Fischer F344 rat urine was purchased from Bioreclamation, Inc. (Westbury, NY). Cellulose dialysis tubing (MWCO 12kD), carboxymethyl cellulose, bovine serum albumin, ammonium sulfate, carbonate-bicarbonate buffer, ammonium acetate, QuantiPro™ BCA Assay Kit, and 3,3′,5,5′-tetramethylbenzidine (TMB) were all purchased from Sigma-Aldrich (St. Louis, MO). Mouse monoclonal anti-rat alpha 2-microglobulin antibody IgG1, goat polyclonal anti-rat alpha 2-microglobulin antibody, and donkey anti-goat IgG horseradish peroxidase (HRP) conjugated antibody were purchased from R&D Systems (Minneapolis, MN).

Purification of alpha 2-microglobulin was performed, with some modifications, essentially as described by (1) as commercial sources were unavailable. Briefly, male rat urine from Fischer F344 rats was kept frozen at -20°C until use. Urine was filtered through a No. 1 Whatman filter and a 75% saturated $(NH_4)_2SO_4$ solution made. The precipitate was dissolved in 0.05 M NH_4OAc (pH5.0) and subsequently dialyzed with the same buffer overnight. The dialysate was placed on a carboxymethyl cellulose column and washed with 0.05 M NH_4OAc (pH = 5.0). Elution buffer consisted of 0.2 M NH_4OAc (pH = 5.0). Fractions were collected and protein determinations (A280) made with a micro-volume spectrophotometer (NanoDrop ND-1000, Thermo Fisher, Wilmington, DE). The major peak fractions were collected and dialyzed against double distilled H_2O for 24 hours. The dialysate was then lyophilized (Advantage XL-70, SP Scientific, Gardiner, NY) according to manufacturer's directions. Samples were resuspended in PBS and protein quantification determined with the QuantiPro™ BCA Assay Kit. Protein samples were then subjected to SDS-PAGE gel electrophoresis and visualized by coomasie staining and by western blotting to confirm the presence of a alpha 2-microglobulin.

Kidneys from experimental animals were removed and one-half of each kidney (left cut longitudinally, right cut transversely) was flash frozen in liquid nitrogen and stored at -80°C until use. Half of each kidney from the same animal were combined and homogenized with a tissue homogenizer in 3 volumes of 1XPBS. Homogenates were then centrifuged at 8000g at 4°C for 10 min and the supernatant centrifuged again at 12,000 g at 4°C for 10 min. Protein quantification of kidney supernatants was determined using a QuantiPro™ BCA Assay Kit. Samples were then aliquoted and stored at -80°C until used.

ELISAs were performed by standard techniques. Briefly, plates were coated with 2 µg/ml of mouse monoclonal anti-rat alpha 2-microglobulin antibody IgG1 for 24-72 hours at 4°C. Plates were then washed with wash buffer (WB) (1X PBS, 0.05% tween-20) 5X. Non-specific binding was reduced by incubating plates with 2% bovine serum albumin (BSA) in WB for 1 hour at room temperature (RT). Subsequently, diluted kidney homogenates were added for 1 hour at RT. Standard curves consisted of purified alpha 2-microglobulin with the highest concentration of 250 ng/ml of alpha 2-microglobulin, followed by 2-fold serial dilutions. After incubation, plates were washed 3X and goat polyclonal anti-rat alpha 2-microglobulin antibody (200 ng/ml) added for 1 hour at room temperature. The plates were then washed 3X and donkey anti-goat IgG horseradish peroxidase (HRP) (1:60,000) antibody added for 1 hour. The plates were

washed 6X and TMB substrate added for approximately 10 minutes. Reactions were stopped by adding 2N H_2SO_4. Absorbance at 450 nm was determined on an ELISA microplate spectrophotometric plate reader (VersaMax, Molecular Devices Sunnyvale, CA).

RESULTS

Measurements of alpha 2-microglobulin protein from the individual animals were used to calculate average and SEM for each group and for both sexes (Table 1). The alpha 2-microglobulin protein amounts were reported as a mass of alpha 2-microglobulin per mass of total protein (giving micrograms alpha 2-microglobulin per milligrams of total protein). This data was also plotted as bar graphs (Figure 1).

An analysis of variance was conducted to determine statistical significance (SYSTAT software, version 13, Inc., Chicago, IL). The data were tested for normality and equivalence of variance. The ANOVA indicated significance and Dunnett's Test was used to compare the test groups with control.

The alpha 2-microglobulin concentration in females exposed at any concentration to HRJ was not significantly different from controls. Female rats do not synthesize alpha 2-microglobulin in the liver as do male rats. Hence, levels of alpha 2-microglobulin in females were approximately two orders of magnitude lower than in males, and there was no dose response related to HRJ inhalation.

The alpha 2-microglobulin concentration in males exposed at the intermediate (700 mg/m^3) and high (2000 mg/m^3) exposure concentrations were significantly different from controls ($p<0.05$). Control levels averaged 54.8 μg/mg total protein, while the low concentration group increased to 75.2 (though not statistically different), and the intermediate group was at 80.5 μg/mg and the high exposure group was at 80.9 μg/mg. There was a slight dose-response of alpha 2-microglobulin concentration as the exposure concentration increased. However, the response was not very strong. For this study, the HRJ was a weak inducer of αμ-globulin accumulation in the F344 male rat.

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Swenberg, J. A. (1993). alpha 2-microglobulin Nephropathy: Review of the cellular and molecular mechanisms involved and their implications for human risk assessment. Environ. Health Perspect. Supplements 101 (Suppl. 6): 39-44.

U.S. EPA. (1991). Alpha 2u-Globulin: Association with chemically induced renal toxicity and neoplasia in the male rat. EPA/a625/3/91/01F.

Table 1. Group average alpha 2-microglobulin levels in kidney samples from HRJ fuel exposed rats. Values reported as μg/mg total protein

	Male	Female
Control	54.8±4.3	0.6±0.2
Low	75.2±6.7	0.3±0.1
Intermediate	80.5±8.3 *	0.2±0.1
High	80.9±5.7 *	0.5±0.2

Values reported as average ±SEM
*Significant ($p < 0.05$) difference from control group

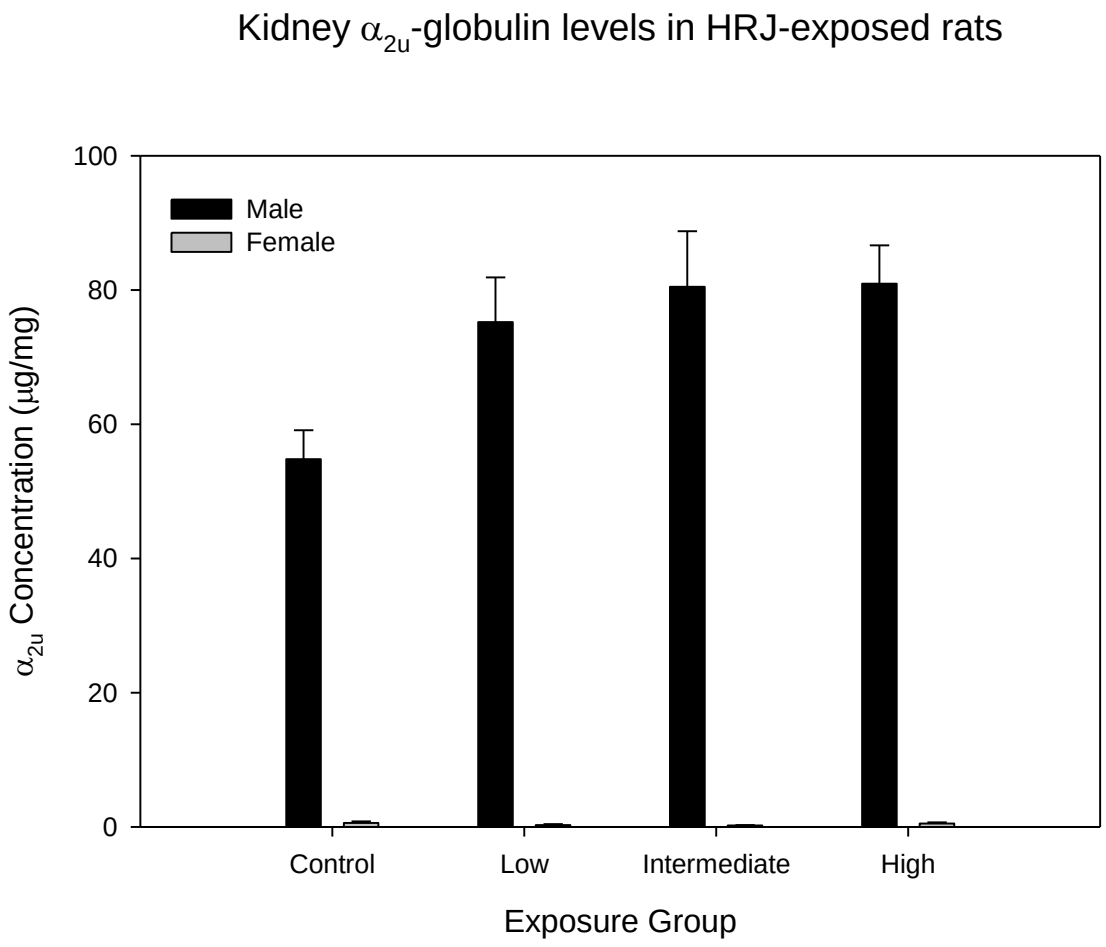

Figure 1. Levels of alpha 2-microglobulin protein in kidney samples from male and female rats exposed by inhalation to HRJ fuel. N=10 per bar, error bars represent ±SEM.

Table 2. Individual alpha 2-microglobulin data

Animal #	Sex	Exposure	TOTAL PROTEIN Adjusted protein concentration (mg/ml)	Adjusted Concentration alpha 2-microglobulin (µg/mL)	µg alpha 2-microglobulin /mg total protein
2	F	Control	31.06	4.37	0.14
4	F	Control	26.66	44.17	1.66
6	F	Control	31.31	6.00	0.19
10	F	Control	29.20	38.70	1.33
12	F	Control	29.45	4.01	0.14
14	F	Control	21.08	6.58	0.31
16	F	Control	30.44	4.99	0.16
18	F	Control	28.04	5.74	0.20
20	F	Control	22.50	38.40	1.71
66	F	Control	26.22	4.20	0.16
8	F	High	20.87	11.54	0.55
62	F	High	29.02	6.80	0.23
64	F	High	54.16	5.98	0.11
68	F	High	17.34	34.00	1.96
70	F	High	28.76	17.21	0.60
72	F	High	29.59	6.71	0.23
74	F	High	21.49	7.40	0.34
76	F	High	31.95	7.25	0.23
78	F	High	32.67	15.82	0.48
80	F	High	29.54	4.84	0.16
22	F	Low	33.60	4.02	0.12
24	F	Low	25.75	3.49	0.14
26	F	Low	26.16	3.37	0.13
28	F	Low	27.57	5.83	0.21
30	F	Low	29.03	44.04	1.52
32	F	Low	24.77	2.34	0.09
34	F	Low	24.12	2.64	0.11
36	F	Low	24.04	2.42	0.10
38	F	Low	18.60	0.97	0.05
40	F	Low	25.68	3.93	0.15
42	F	Medium	16.40	3.84	0.23
44	F	Medium	24.71	3.55	0.14
48	F	Medium	23.43	2.82	0.12

Table 2. Individual alpha 2-microglobulin data (continued)

50	F	Medium	23.21	3.65	0.16
52	F	Medium	28.35	4.54	0.16
54	F	Medium	17.77	10.88	0.61
56	F	Medium	24.08	6.16	0.26
58	F	Medium	29.95	6.37	0.21
60	F	Medium	25.06	2.91	0.12
1	M	Control	27.18	1410.28	51.88
3	M	Control	25.84	954.98	36.96
5	M	Control	27.11	1179.00	43.49
7	M	Control	36.82	2827.24	76.78
9	M	Control	37.80	1584.83	41.92
11	M	Control	23.25	1778.42	76.49
13	M	Control	25.00	1278.09	51.13
15	M	Control	21.77	1076.37	49.45
17	M	Control	26.33	1602.82	60.87
19	M	Control	33.29	1961.12	58.91
63	M	High	31.41	1771.24	56.40
65	M	High	27.64	2724.05	98.55
67	M	High	29.99	2512.96	83.78
69	M	High	29.51	1778.04	60.24
71	M	High	41.93	2774.09	66.17
73	M	High	28.87	2510.31	86.96
75	M	High	42.48	4167.06	98.10
77	M	High	31.86	3237.44	101.60
79	M	High	28.97	2219.71	76.62
21	M	Low	23.66	812.28	34.33
23	M	Low	33.01	3630.38	109.97
25	M	Low	30.17	2143.49	71.05
27	M	Low	27.52	1736.66	63.12
29	M	Low	28.52	2045.66	71.72
31	M	Low	28.26	2511.10	88.85
33	M	Low	18.34	1681.33	91.69
35	M	Low	20.76	1452.41	69.95
37	M	Low	34.89	2110.55	60.49
39	M	Low	30.56	2779.64	90.96
41	M	Medium	28.03	3638.79	129.83
43	M	Medium	38.20	3241.82	84.87
45	M	Medium	33.61	1759.64	52.36

47	M	Medium	29.32	1669.25	56.94

Table 2. Individual alpha 2-microglobulin data (continued)

49	M	Medium	28.13	1959.28	69.65
53	M	Medium	30.61	2646.54	86.46
55	M	Medium	26.38	1581.23	59.94
57	M	Medium	34.66	3825.65	110.37
59	M	Medium	45.83	2987.47	65.18
51	M	Medium	23.27	2071.78	89.04

APPENDIX J. EVALUATION OF GENOTOXICITY BY MEASUREMENT OF MICRONUCLEI IN BONE MARROW SAMPLES FROM F344 RATS EXPOSED BY INHALATION TO HEFA-C FUEL

Study Title

90-Day Inhalation Toxicity Study of HEFA-C (HRJ) Fuel in Rats (Rattus norvegicus) with Neurotoxicity Testing and Genotoxicity Assay

Study Protocol

F-WA-2011-0126-A

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Introduction

A Hydrotreated Renewable Jet (HRJ) fuel produced from an extract of the camelina plant is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the US armed forces. During fueling operations, personnel may be exposed to vapors and aerosols of jet fuel by inhalation. A study was performed to assess the potential toxicity of HRJ fuel by inhalation. F344 Rats were exposed by inhalation to an aerosol and vapor mixture of FT Jet Fuel with additives. Whole body inhalation exposures were conducted 6 hours/day, 5 days/week over a 90-day period, at concentrations of 0 (control), 200, 700, or 2000 mg/m^3. Groups of 10 males and 10 females were exposed at each exposure concentration for a total of 40 males and 40 females.

An ancillary experiment was conducted to assess the genotoxic potential of inhaled HRJ fuel. A sub-group of F344 rats was exposed for two weeks to the aerosol and vapor mixture of HRJ fuel (concurrent with the experiment described above) to conduct a genotoxicity assay based on the US EPA Health Effects Test Guidelines, OPPTS 870.5395, "Mammalian Erythrocyte Micronucleus Test". In this study, 5 males and 5 females per exposure concentration (total of 40 animals) were exposed to the HRJ fuel. An additional 10 males and 10 females, not exposed to jet fuel, were administered positive and vehicle controls, for a total of 60 animals for the micronucleus assay. At the end of exposures, animals were euthanized and dissected to extract bone marrow from the femur. The bone marrow was processed and analyzed by flow cytometry for reticulocytes and micronucleated reticulocytes, an indicator of genotoxicity.

Methods

This genotoxicity study is based on the U.S. Environmental Protection Agency (U.S. EPA) Harmonized Test Guideline developed by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 870 870.5395 "Mammalian Erythrocyte Micronucleus Test". During the conduct of the primary 90-day inhalation exposure study, an ancillary study of F344 rats (5 males and 5 females per exposure concentration, total of 40 animals) were exposed to the same concentrations of HRJ fuel for approximately 2 weeks (10 exposures) for a bone marrow micronucleus assay to assess potential genotoxicity. An additional 10 males and 10 females, not exposed to jet fuel, were used as positive and vehicle controls, for a total of 60 animals for the micronucleus assay (Table 1). Animals in the micronucleus study were euthanized by IP injection of sodium pentobarbital, and dissected to remove the femur. Bone marrow was isolated by flushing the femur with 1 ml of heat-inactivated fetal bovine serum (FBS) into ice-cold 100% methanol fixative. Bone marrow cells were then processed, stained and analyzed by flow cytometry according to the manufacturer's instructions (MicroFlow Plus RBM, Litron Laboratories, Rochester NY) and Fiedler *et al.*, (2010).

Table 1: Two-week Inhalation of HRJ Fuel in Rats with Genotoxicity Assay

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control	0	5	5
Low	200	5	5
Intermediate	700	5	5
High	2000	5	5
Negative Control	Saline	5	5
Positive Control	CP	5	5
Total		30	30

Table 2: Timeline

Date	Activity
June 17, 2011	Animals ordered
June 28, 2011	Animals arrive, placed in room nn
July 05, 2011	Animals begin cage acclimation
July 13, 2011	Exposures begin for micronucleus cohort
July 28, 2011	Exposures end for micronucleus cohort
	Necropsy

Micronuclei Observation:

The frequency of micronucleated cells was measured by flow cytometry from approximately 20,000 reticulocytes per animal. The percentage of reticulocytes (%RET), micronucleated mature normochromatic erythrocytes (%MN-NCE), and micronucleated reticulocytes (%MN-RET) were determined per animal. The results of the micronucleus assay could be considered positive if there was: a clear dose-related increase in the number of micronucleated reticulocytes; or a reproducible and statistically significant increase in the micronucleated reticulocyte frequency was detected for at least one concentration of the test substance.

Results

For the positive control study, the percentage of reticulocytes in the cyclophosphamide-treated animals decreased compared with the vehicle controls (Table 3). The difference was statistically significant, and indicative of an expected response to the toxic CP. By contrast, the number of micronucleated reticulocytes was about the same between the vehicle control and the CP-treated animals (Table 3). It was expected that the number of micronucleated cells would increase significantly in the CP-treated animals. The reason for this unexpected finding is unknown but may be related to time course of the development of micronucleated reticulocytes. When CP is administered intraperitoneally, the number of MN starts to develop after 1 day, and reaches a peak approximately a few days after the last administration of CP. For future studies with CP, it appears that the treatment should go for a longer period of time.

The animals that had undergone a 2 week exposure to HRJ fuel showed no significant difference in the percentage of reticulocytes compared with air-exposed controls. Therefore, there was no apparent toxicity to the reticulocytes caused by exposure to HRJ fuel. The exposed animals did show a slight difference in the percentage of micronucleated reticulocytes, however this difference was not dose-related. The low and high exposure groups were at approximately the same percentage, while the intermediate exposure group was lower than controls. A statistical analysis (ANOVA with Holm Sidak method of pairwise comparisons) did not indicate a statistically significance between all possible pairs. Thus, there was no evidence of genotoxicity, as identified by micronucleated cells, in animals exposed to HRJ fuel by inhalation.

References

Fiedler, R. D., Weiner, S. K., and Schuler, M. (2010). Evaluation of a modified CD71 MicroFlow® method for the flow cytometric analysis of micronuclei in rat bone marrow erythrocytes. Mutation Research 703 (2010) 122–129.

Table 3. Percentage of reticulocytes and micronucleated reticulocytes in genotoxicity study animals

Animal No.	Sex	%RET	%MN-RET
81	M	19.43	0.9
82	F	10.1	0.79
83	M	13.83	0.9
84	F	13	0.77
85	M	20.26	0.96
86	F	12.21	0.78
87	M	18.15	0.69
88	F	15.17	0.59
89	M	16.26	1.01
90	F	26.08	0.77
91	M	42.4	0.47
92	F	19.55	0.56
93	M	21.71	0.62
94	F	30.58	1.13
95	M	40.92	0.66
96	F	20.98	0.81
97	M	23.64	0.51
98	F	31.6	1.68
99	M	31.98	0.9
100	F	25.03	0.66
101	M	18.04	0.19
102	F	24.07	0.23
103	M	45.72	0.37
104	F	21.95	1.2
105	M	33.27	0.66
106	F	28.68	0.35
107	M	44.43	0.8
108	F	34.4	1.09
109	M	24.67	0.26
110	F	25.53	0.9
111	M	22.56	0.33
112	F	38.76	1.33
113	M	42.94	0.57
114	F	20.97	1.53
115	M	39.66	0.49

Table 3. Percentage of reticulocytes and micronucleated reticulocytes in genotoxicity study animals (continued)

116	F	32.81	0.52
117	M	17.92	1.09
118	F	31.88	0.93
119	M	28.52	1.05
120	F	27.66	1.43
121	M	44.02	0.43
122	F	33.5	0.25
123	M	33.6	0.88
124	F	42.13	0.65
125	M	23.9	0.92
126	F	39.94	0.33
127	M	19.99	0.3
128	F	24.89	0.7
129	M	20.94	0.41
130	F	29.64	0.37
131	M	20.04	0.4
132	F	20.85	1.77
133	M	32.88	1.23
134	F	30.01	0.85
135	M	26.05	0.84
136	F	39.93	0.36
137	M	51.95	1.52
138	F	49.75	1.15
139	M	40.73	0.83
140	F	40.33	0.95

Table 4. Cell count data in genotoxicity animals

		Cell Counts			
Animal No.	**Group**	**NCE**	**MN-NCE**	**RET**	**MN-RET**
81	Vehicle Control	16105	12	3852	35
82	Vehicle Control	17974	7	2005	16
83	Vehicle Control	17228	9	2741	25
84	Vehicle Control	17390	13	2581	20
85	Vehicle Control	15940	5	4012	39
86	Vehicle Control	17549	7	2423	19
87	Vehicle Control	16359	4	3604	25
88	Vehicle Control	16958	5	3016	18
89	Vehicle Control	16743	5	3219	33
90	Vehicle Control	14776	7	5175	40
91	Positive Control	11486	35	8441	40
92	Positive Control	16071	24	3888	22
93	Positive Control	15642	16	4316	27
94	Positive Control	13827	62	6048	69
95	Positive Control	11792	19	8127	54
96	Positive Control	15778	16	4160	34
97	Positive Control	15253	16	4704	24
98	Positive Control	13633	26	6203	106
99	Positive Control	13400	19	6252	57
100	Positive Control	14978	16	4973	33
101	Air Control	16385	8	3601	7
102	Air Control	15182	7	4804	11
103	Air Control	10844	14	9112	34
104	Air Control	10922	8	3036	37
105	Air Control	13331	13	6609	44
106	Air Control	14259	3	5716	20
107	Air Control	11077	35	8815	71
108	Air Control	13073	46	6803	75
109	Air Control	15058	7	4921	13
110	Air Control	14883	12	5061	46
111	Low Concentration	15484	8	4497	15
112	Low Concentration	12203	49	7652	103
113	Low Concentration	11402	12	8542	49
114	Low Concentration	15780	33	4131	64
115	Low Concentration	12050	12	7889	39

Table 4. Cell count data in genotoxicity animals (continued)

116	Low Concentration	13426	7	6526	34
117	Low Concentration	16402	15	3544	39
118	Low Concentration	13598	24	6317	59
119	Low Concentration	14271	23	5644	60
120	Low Concentration	14444	25	5452	79
121	Intermediate	11190	7	8767	38
122	Intermediate	13300	2	6683	17
123	Intermediate	13265	7	6657	59
124	Intermediate	11541	17	8361	55
125	Intermediate	15207	6	4733	44
126	Intermediate	11999	10	7960	26
127	Intermediate	15995	6	3986	12
128	Intermediate	15011	12	4942	35
129	Intermediate	15795	18	4170	17
130	Intermediate	14060	13	5905	22
131	High Concentration	15989	7	3994	16
132	High Concentration	15791	42	4097	74
133	High Concentration	13350	66	6491	81
134	High Concentration	13963	28	5947	51
135	High Concentration	14773	14	5164	44
136	High Concentration	12002	8	7954	29
137	High Concentration	9560	47	10229	158
138	High Concentration	10013	36	9835	114
139	High Concentration	11828	26	8078	68
140	High Concentration	11897	37	7989	77

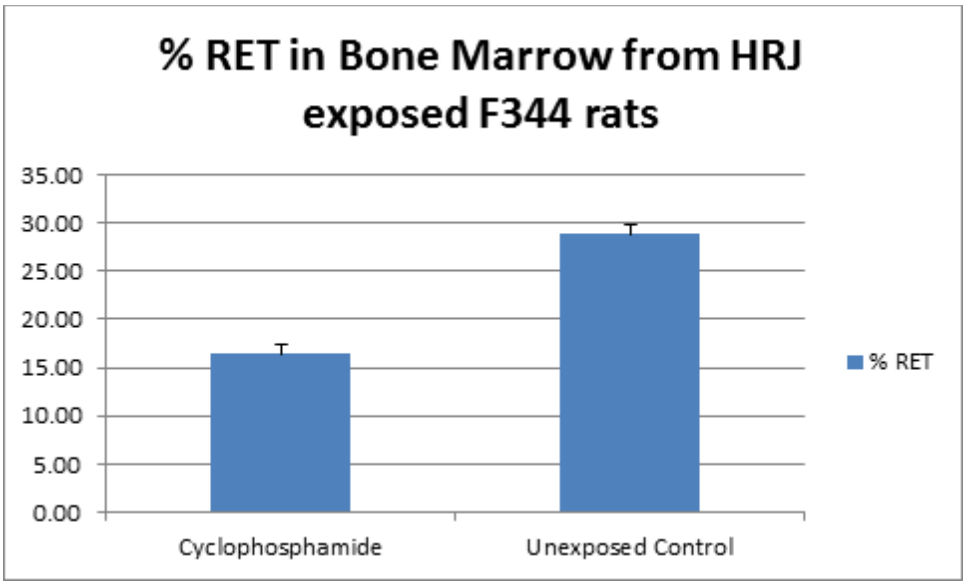

Figure 1. Reticulocytes in cyclophosphamide treated rats

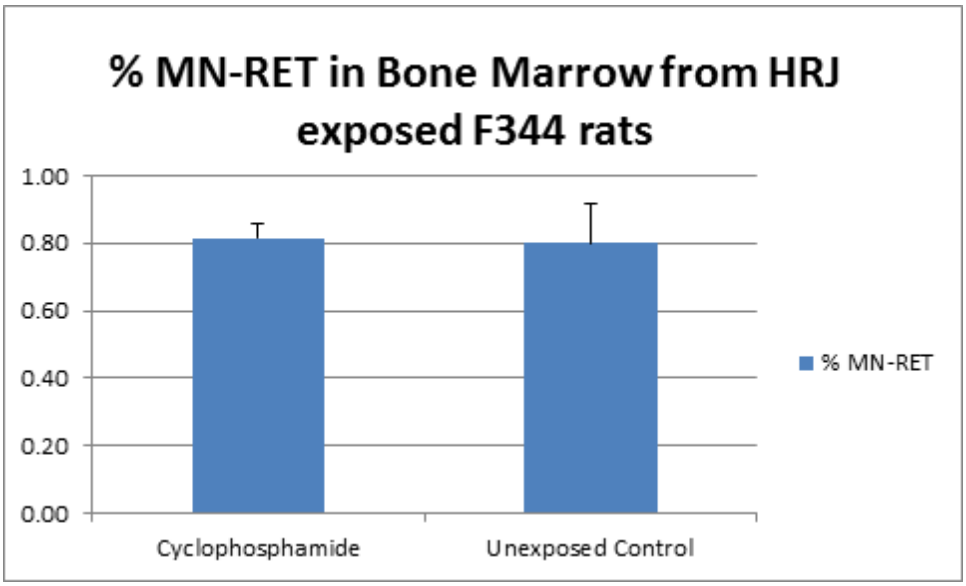

Figure 2. Micronucleated reticulocytes in cyclophosphamide treated rats

LIST OF ACRONYMS

°C	degrees Celsius
µg	microgram
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AFB	Air Force Base
ANOVA	analysis of variance
cm	centimeter
DTIC	Defense Technical Information Center
dL	deciliter
EPA	Environmental Protection Agency
ELISA	enzyme-linked immunosorbent assay
F-T	Fischer-Tropsch
FOB	functional observational battery
FMI	Fluid Metering, Inc.
FTIR	Fourier transform infrared
g	gram
GLP	Good Laboratory Practices
GSD	geometric standard deviation
HEFA	hydroprocessed esters and fatty acids
HEFA-C	HEFA-Camelina
HEFA-F	HEFA-Animal fats and oils
HEFA-T	HEFA-Tallow
HEPA	high efficiency particulate air
HJF	Henry M. Jackson Foundation for the Advancement of Military Medicine
HRJ	hydrotreated renewable jet
IACUC	Installation Animal Care and Use Committee
INR	international normalized ratio
kg	kilogram
LOAEL	lowest observed adverse effect level
m^3	cubic meter
MMAD	mass median aerodynamic diameter
mg	milligram
min	minutes
mL, ml	milliliter
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PAS	photobeam activity system
PT	prothrombin time
sec	seconds
SOP	standard operating procedure
SPK	Synthetic paraffinic kerosene
THRU	Toxic Hazard Research Unit